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EFFECT OF FILTRATION ON THE SANITARY QUALITY OF THE WATER OF THE METROPOLITAN WATER DISTRICT

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TWO TEXT FIGURES

INTRODUCTION

A filter plant for a public water-supply system has two principal purposes; namely, real, to improve the quality of the water for drinking purposes; and psychological, to inspire and develop public confidence. Just how far the new filter plant of the City of Manila has accomplished the first purpose is the object of our present study. As to the second it can be stated that even at this early period, a large part of our public has begun to utilize the city water for drinking uses with greater confidence.

In a previous investigation,(1) made when the city water supply was not filtered, we showed that the sanitary quality of the water was not always satisfactory at certain times of the year and that chlorination alone was not sufficient to reduce effectively the number of bacteria and to eliminate organisms of the *Bacillus coli-aërogenes* group. We then took the occasion to point out that filtration in addition to chlorination seemed to be the most feasible means of improving the sanitary quality of the water. We were, therefore, very much pleased to find that the Governor-General and other public officials shared the same views and used their good offices in bringing about the immediate completion of the filter plant.

In May, 1935, the filters were operated for the first time, but since the filters were new and many experiments had to be conducted to adjust the dose of chemicals or correct small errors in construction, the first months of filtration should really be considered as a period of experimentation and we have designated it as such in this paper. It was not until July, or two months after, that the filters actually started to function normally and to operate effectively.

The filters of the Metropolitan Water District are of the rapid sand (mechanical gravity) type. The filter plant is located at the junction of the new Novaliches and the old Montalban lines at the barrio of Balara, municipality of Marikina, and at an elevation above the pipe line from Montalban but below that of Novaliches. Consequently, the Novaliches water enters the filter plant by gravity, but the Montalban water is lifted by pumps. The main parts of the filter plant in the order of the flow of water are the following: Two chemical houses where the filter alum is added; a long mixing chamber provided with numerous baffles to assure sufficient agitation of the water (the Montalban water does not pass through this chamber); two coagulating or settling basins where part of the "floc" settles; eight filter units of two beds each with a total area of 1,296 square meters; and an open aërator tank where the filtered water is exposed for aëration through nozzles like a series of fountains. The filter plant was designed for a capacity of 40 million gallons per day. It was constructed at a total approximate cost of 1,300,000 pesos and will cost about 120,000 pesos annually to operate.

The filtered water finally passes through an uncovered distributing reservoir located in the municipality of San Juan, 5 kilometers distant, where 0.4 part per million of liquid chlorine is applied before final distribution. However, June 23, 1935, another chlorinator was installed in the filter plant at the outlet of the aërator tank. After this date the dose of chlorine was split into two halves, and 0.2 part per million was applied at the filter plant and 0.2 part per million was added in San Juan.

PROCEDURE OF INVESTIGATION

From September, 1931, to November, 1934, the collection of samples was made on an average of once a week for the raw water of Novaliches and three times a week for the tap water in the city. We intended to collect more frequently, but the

pressure of other duties and the lack of sufficient materials prevented us from doing so. For the same reason we were unable to make the completed tests for the *coli-aërogenes* group of organisms, and reported only the results of presumptive tests after twenty-four hours.

However, in November, 1934, through arrangements made by the Metropolitan Water District, ample help, supplies, and materials were made available for a more intensive study. Therefore, from this date to October, 1935, a period of about one year, a large number of samples from different parts of the metropolitan water system were brought to our laboratory for examination.

During this latter period samples of water were collected twice a day from a tap at the School of Hygiene, and once daily from the different parts of the filter plant, the raw water from Novaliches and Montalban, the San Juan reservoir, and several taps in San Juan, Santa Mesa, Santa Ana, and Tondo. From the other districts of Manila and suburban towns samples were taken twice a week.

In the routine analysis of the sample, the following examinations were included: Bacterial counts per cubic centimeter on agar plates incubated for twenty-four hours at 37° C., tests for organisms of the *coli-aërogenes* group, and measurements of turbidity and residual chlorine. All examinations were made in conformity with the Standard Methods of Water Analysis recommended by the American Public Health Association.(2)

In the tests for the *coli-aërogenes* group we inoculated five 10-cc portions of each sample to lactose-broth fermentation tubes as required in the Drinking Water Standards of the United States Treasury Department Public Health Service.(3) Due to the additional help at our disposal the examinations for the organisms of the *coli-aërogenes* group were performed up to the completed tests. Differentiation of fæcal and nonfæcal organisms was even attempted as time permitted, for which the uric acid and sodium citrate media were employed. It should be mentioned, however, that these differentiation tests are not called for in the bacteriological requirements of the United States Treasury Department standards.

We also made microscopical examinations of the Novaliches raw water and the filtered tap water following the Sedgewick-Rafter method described in the Standard Methods of the American Public Health Association.

RESULTS OF INVESTIGATION

In Table 1 we classified the results of our examinations by months covering the period from November, 1934, to October, 1935, inclusive. We divided the different locations from which samples were collected into eleven groups, as follows: Montalban reservoir, Novaliches reservoir, Balara settling basins, Balara filter effluent, Balara surge tank, Balara aërator, San Juan reservoir, School of Hygiene tap, other city taps, chlorinated suburban taps, and suburban taps receiving only a half dose of chlorine. It must be explained in this connection that the last-mentioned suburban taps received only 0.2 part per million of chlorine from the first chlorinator at the filter plant. This group, however, comprises only a small area in San Juan where the pipe line was tapped above the second chlorinator.

Table 1 also includes a summary of all monthly observations. The average bacterial counts and the percentage positives for the *coli-aërogenes* group of this summary are plotted in text figs. 1 and 2, respectively.

In Table 2 is recorded a summary of the results of the examinations of the city water from September, 1931, to November, 1934, previous to the period covered in Table 1. These were placed in a separate table because of the fewer samples collected and because the examination for the *B. coli-aërogenes* organisms included only presumptive tests after twenty-four hours.

Tables 1 and 2 and text figs. 1 and 2 show that the city water before the completion of the filters, despite continuous disinfection with 0.4 to 0.6 part per million of liquid chlorine, was not always satisfactory for drinking purposes due to the frequent occurrence of organisms of the *coli-aërogenes* group and the irregular bacterial content. However, taking the total of all observations the bacterial counts reached over 100 per cc in only 215, or 18 per cent, of the total of 1,155 samples, and the tests for organisms of the *coli-aërogenes* group were positive in 119, or 2.4 per cent, of 4,859 portions. Although these total percentages for *coli-aërogenes* are low when considered on the basis of the United States Treasury standards, their irregular distribution made the water suspicious at certain months of the year, especially during the rainy season. For example, in November, 1934, 23 per cent of the tests from the School of Hygiene tap and 40 per cent from suburban taps gave positive *coli-aërogenes* tests, thus failing to meet the bacteriological re-

quirements of the United States Treasury Department standards during this particular month.

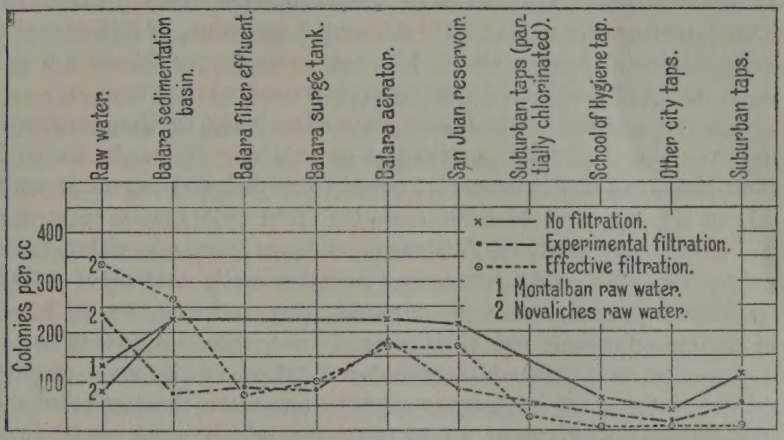

FIG. 1. Average number of bacteria per cubic centimeter in samples collected from different parts of the Metropolitan water system.

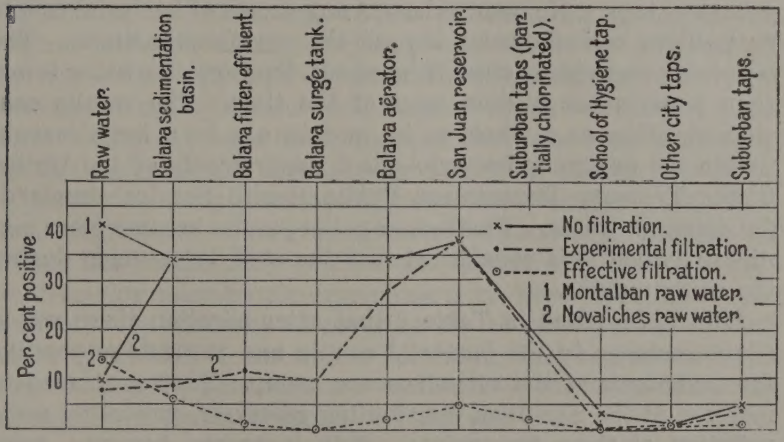

FIG. 2. Percentage positive for *coli-aërogenes* test of samples collected from different parts of the Metropolitan water system.

Furthermore, the turbidity of unfiltered water could not be removed and was quite high during rainy months. It is probable that the poor quality of the Montalban source contributed to the inferior quality of the water before filtration. Table 2 corroborates the findings in Table 1.

The improvement of the city water started even during the experimental filtration period, as shown by the diminution in bacterial counts and positive *coli-aërogenes* tests (Table 1). Thus, during this period only 54, or 9.1 per cent, of 596 samples gave bacterial counts above 100 per cc and only 46, or 1.6 per cent, of 2,962 portions gave positive *coli-aërogenes* tests.

When the filters started to operate effectively, further improvement in the city water is readily noticeable. Thus, in Table 1 it is shown that the bacterial count exceeded 100 per cc in only 15, or 1.1 per cent, of 1,306 samples from chlorinated taps, and 17, or 6.8 per cent, of 249 samples from partially chlorinated suburban taps. The remaining samples with a total of 1,291, or 98.9 per cent, from chlorinated taps and 232, or 93.2 per cent, from partially chlorinated taps gave bacterial counts below 100 per cc, certainly an excellent indication of the good quality of the water. Of even more significance were the tests for the *coli-aërogenes* group in 10 cc portions, which were positive in only 22, or 0.3 per cent, of 6,422 tubes from chlorinated taps; and 5, or 1.9 per cent, of 269 tubes from partially chlorinated suburban taps. No month showed any excess of bacterial counts or positive *coli-aërogenes* beyond the permissible limits. The tap water was visibly clear throughout, the turbidity being lower than 5 parts per million most of the time. The results considered either as a whole or by months are by a large margin within the minimum bacteriological requirements of the United States Treasury Department Public Health Service standards for drinking water. Furthermore, the results become more significant when it is considered that the study was made during the rainy months.

It is to be noted in Table 1 that after aëration there was a slight increase in the bacterial counts and percentage positive for organisms of the *coli-aërogenes* group. They continued to increase at the San Juan distributing reservoir, indicating some pollution at these two points. Both increases, however, were completely overcome by chlorination.

It is interesting to observe the close correlation between the bacterial counts and the tests for the *coli-aërogenes* group. This is more clearly shown in the text figures where parallelism between the curves of the two tests can be readily seen. Thus, low counts were usually accompanied with low percentage positive for the *coli-aërogenes* group and high counts with high percentage positive for *coli-aërogenes*.

Under residual chlorine in Table 1 it will be seen that by and large the residual chlorine found in different parts of the city, except at the School of Hygiene, falls within the desirable limits required by present-day practices. We presume that the failure of the School of Hygiene tap and most of the suburban taps to reach even the minimum limit is due to some peculiarities unknown to us in the layout of the pipe lines. However, the half dose of chlorine given to a few suburban taps already mentioned elsewhere accounts for the findings of very little residual chlorine at these places.

Our microscopical examination of raw water from the Novaliches reservoir, made during December, 1935, revealed the presence of plenty of microörganisms, with an average of 285 per cc. These organisms included plants of the families Chlorophyceæ, Cyanophyceæ, and Diatomaceæ, with predominance of the last group; and protozoan and crustacean animals, of which the protozoan were the more numerous. Cox, Heise, and Gana(4) in similar examinations made in 1913 also reported large numbers of microörganisms in the unfiltered city tap water. It is significant, therefore, that in the examinations of filtered tap water, made on dates coinciding with the same examinations of raw water, only an average of fourteen organisms per cubic centimeter could be recovered, most of which were diatoms. It is probable that these few organisms developed during exposure of the water at the open aërator and brief storage in the uncovered San Juan distributing reservoir where an abundance of sunlight favored the growth and reproduction of the plankton. While our results may point to the efficiency of sand filtration in removing microscopic organisms from water supplies, it also indicates the necessity of covering the aërator and distributing reservoir, which in addition to removing these organisms would prevent possible pollution from malicious or inadvertent sources, especially in times of public disorder and war.

SUMMARY AND CONCLUSION

1. Our study verifies again the superiority of the Novaliches raw water over that of Montalban from the bacteriological point of view, as pointed out in a previous paper.

2. The unfiltered water of either Novaliches or Montalban, even if disinfected with 0.4 to 0.6 part per million of chlorine, was not always satisfactory with regards to its sanitary quality.

3. The filtration of Novaliches water since July, 1935, has markedly improved the quality of the metropolitan water as

shown by low bacterial counts and negative tests for *Bacillus coli-aërogenes* organisms.

4. Close relationship between the colony counts and the tests for *coli-aërogenes* organisms was noted.

5. An increase both in colony counts and percentage positive for *coli-aërogenes* of the filtered water was observed at the open aërator tank and the uncovered San Juan distributing reservoir, which was, however, overcome by chlorination.

6. After filtration the tap water showed no visible turbidity.

7. Microörganisms present in abundance in raw water were markedly reduced in the filtered tap water. These few remaining organisms can probably be removed by covering the aërator and the distributing reservoir.

8. From the results of four months' study of the effects of rapid sand filtration it may be concluded that the city water fully conforms to the standards of the United States Treasury Department Public Health Service for drinking waters. In our opinion the water is safe to drink as long as the Metropolitan Water District keeps up the satisfactory record of performance that it showed during the first four months of filtration.

ACKNOWLEDGMENT

We wish to express our appreciation to Mr. Gregorio Anonas, manager, and Mr. Ambrosio Magsaysay, chief engineer, Metropolitan Water District, for facilitating arrangements that made this study possible. To Mr. Guillermo Abdon, of the same office, we are also grateful for valuable assistance. Finally, we are thankful to our colleagues of the School of Hygiene and Public Health for kindly suggestions.

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TABLE 1.—*Monthly records of laboratory examinations of the Metropolitan Water District water from November, 1934, to October, 1935, showing the effects of filtration.*

BEFORE FILTRATION.

Date.	Location.	Samples.	Average turbidity.	Average residual chlorine.	Colonies per cc after 24 hours at 37° C.				Test for *coli-aërogenes* group.			
									10-cc portion.		1-cc portion.	
					Mean.	<100.	>100.	>1,000.	Number.	Positive.	Number.	Positive.
			p. p. m.	*p. p. m.*		*P. ct.*	*P. ct.*	*P. ct.*		*P. ct.*		*P. ct.*
November, 1934	Montalban raw	1			192	0	100	0	5	100		
Do	Balara raw	27			174	41	59	0	103	49		
Do	San Juan reservoir	4			122	50	50	0	20	60		
Do	School of Hygiene tap	28	1.74	0.016	75	82	18	0	60	23		
Do	Other city taps	12		0.018	11	100	0	0	12	0		
Do	Suburban taps	25		0.012	102	64	36	0	25	40		
December, 1934	Montalban raw	7			161	29	71	0	33	100		
Do	Novaliches raw	1			42	100	0	0	3	100		
Do	Balara raw	31			525	52	48	10	150	50		
Do	San Juan reservoir	22			72	71	29	0	103	79		
Do	School of Hygiene tap	41	3.17	0.038	12	100	0	0	205	10		
Do	Other city taps	45		0.292	8	100	0	0	49	6		
Do	Suburban taps	35		0.046	30	89	11	0	35	20		
January, 1935	Montalban raw	21			90	67	33	0	70	46		
Do	Novaliches raw	15			72	73	27	0	39	8		
Do	Balara raw	14			73	86	14	7	70	7		
Do	San Juan reservoir	42			76	90	10	0	138	15		
Do	School of Hygiene tap	49	1.80	0.175	5	100	0	0	245	0		
Do	Other city taps	115		0.422	6	99	1	0	327	0		
Do	Suburban taps	44		0.178	29	93	7	0	136	1		
February, 1935	Montalban raw	23			88	70	30	0	43	33	35	6
Do	Novaliches raw	24			58	87	13	0	24	17	15	7
Do	Balara raw	3			68	67	33	0	3	0	3	0
Do	San Juan reservoir	46			120	54	46	0	50	28	34	12

TABLE 1.—*Monthly records of laboratory examinations of the Metropolitan Water District water from November, 1934, to October, 1935, showing the effects of filtration*—Continued.

BEFORE FILTRATION—Continued.

Date.	Location.	Samples.	Average turbidity.	Average residual chlorine.	Colonies per cc after 24 hours at 37° C.				Test for *coli-aërogenes* group.			
									10-cc portion.		1-cc portion.	
					Mean.	<100.	>100.	>1,000.	Number.	Positive.	Number.	Positive.
			p. p. m.	*p. p. m.*		*P. ct.*	*P. ct.*	*P. ct.*		*P. ct.*		*P. ct.*
February, 1935	School of Hygiene tap	45	2.23	0.073	44	91	9	0	225	0	165	0
Do	Other city taps	126		0.321	20	94	6	0	625	0.3		
Do	Suburban taps	60		0.076	71	75	25	0	280	1		
March, 1935	Montalban raw	25			132	76	24	0	121	25	121	3
Do	Novaliches raw	24			64	87	17	0	25	3	25	0
Do	San Juan reservoir	50			153	62	38	0	50	32	50	6
Do	School of Hygiene tap	50	2.92	0.056	148	56	44	0	250	0	250	0.4
Do	Other city taps	147		0.158	82	67	33	0	735	4		
Do	Suburban taps	61		0.046	194	44	56	2	305	4		
April, 1935	Montalban raw	6			380	33	67	17	13	23	13	8
Do	Novaliches raw	14			170	71	29	7	14	0	14	0
Do	San Juan reservoir	34			755	35	65	6	89	34	89	8
Do	School of Hygiene tap	34	2.50	0.059	60	85	15	0	170	0	170	0
Do	Other city taps	95		0.239	79	76	24	0	455	0		
Do	Suburban taps	34		0.086	126	59	41	0	160	5		
May, 1935	Novaliches raw	3			148	67	33	0	3	33	3	0
Do	San Juan reservoir	6			236	0	100	0	18	39	13	0
Do	School of Hygiene tap	6	4.37	0.102	352	17	83	17	30	0	30	0
Do	Other city taps	24		0.217	72	79	21	0	120	0		
Do	Suburban taps	12		0.020	454	25	75	8	60	5		

June, 1935	Novaliches raw	12	2.83		42	100	0	0	17	0	17	0
Do	Balara raw	31			47	97	3	0	78	8	18	0
Do	San Juan reservoir	16			97	75	25	0	48	36	8	25
Do	School of Hygiene tap	12	0.42	0.078	4	100	0	0	60	0		
Do	Other city taps	45	0.17	0.120	9	98	2	0	225	0		
Do	Suburban taps	13	0.00	0.028	32	92	8	0	65	8		

EXPERIMENTAL FILTRATION.

March, 1935	Novaliches raw	2			93	50	50	0	2	0	2	0
Do	Balara sedimentation basin	9			72	78	22	0	13	0	6	0
Do	Balara filter effluent	5			137	60	40	0	25	52	15	0
Do	San Juan reservoir	6			133	50	50	0	6	33	4	25
Do	School of Hygiene tap	3	1.67	0.080	228	0	100	0	15	0	15	0
Do	Other city taps	4		0.262	4	100	0	0	20	0		
Do	Suburban taps	8		0.091	124	50	50	0	40	0		
April, 1935	Novaliches raw	10			508	50	50	10	10	20	10	0
Do	Balara sedimentation basin	32			75	87	13	0	32	19	32	3
Do	Balara filter effluent	8			65	88	12	0	40	47	35	14
Do	Balara aërator	14			269	36	64	7	38	100	38	100
Do	San Juan reservoir	20			132	40	60	0	51	76	51	53
Do	School of Hygiene tap	16	0.02	0.048	124	56	44	0	80	2	80	0
Do	Other city taps	36		0.239	79	83	17	0	180	1		
Do	Suburban taps	30		0.056	105	50	50	0	150	4		
May, 1935	Novaliches raw	42	0.44		255	76	24	2	60	8	60	0
Do	Balara sedimentation basin	83			94	84	16	2	84	11	77	0
Do	Balara filter effluent	142			115	48	52	1	162	6	157	2
Do	Balara surge tank	35	0.00		111	69	31	0	171	19	1	0
Do	Balara aërator	32	0.00		256	23	77	6	152	39	6	17
Do	San Juan reservoir	88			52	83	17	0	264	29	64	2
Do	School of Hygiene tap	49	0.00	0.048	17	96	4	0	241	0	41	0
Do	Other city taps	164	0.10	0.142	8	99	1	0	820	1		
Do	Suburban taps	59	0.17	0.045	68	76	24	0	291	7		

TABLE 1.—*Monthly records of laboratory examinations of the Metropolitan Water District water from November, 1934, to October, 1935, showing the effects of filtration*—Continued.

EXPERIMENTAL FILTRATION—Continued.

Date.	Location.	Samples.	Average turbidity.	Average residual chlorine.	Colonies per cc after 24 hours at 37° C.				Test for *coli-aërogenes* group.			
									10-cc portion.		1-cc portion.	
					Mean.	<100.	>100.	>1,000.	Number.	Positive.	Number.	Positive.
			p. p. m.	*p. p. m.*		*P. ct.*	*P. ct.*	*P. ct.*		*P. ct.*		*P. ct.*
June, 1935	Novaliches raw	37	2.50	----------	146	68	32	3	37	5	37	3
Do	Balara sedimentation basin	62	0.56	----------	52	87	13	0	62	5	62	0
Do	Balara filter effluent	145	----------	----------	56	85	15	0	145	1	145	1
Do	Balara surge tank	36	----------	----------	51	89	11	0	175	1	----------	----------
Do	Balara aërator	32	----------	----------	49	88	12	0	165	2	----------	----------
Do	San Juan reservoir	50	----------	----------	105	78	22	0	114	30	10	----------
Do	School of Hygiene tap	37	0.00	0.076	2	100	0	0	185	0	----------	----------
Do	Other city taps	131	0.00	0.131	6	99	1	0	645	0	----------	----------
Do	Suburban taps	59	0.00	0.036	9	100	0	0	295	2	----------	----------

EFFECTIVE FILTRATION.

July, 1935	Novaliches raw	38	5.74	----------	306	18	82	5	40	30	40	17
Do	Balara sedimentation basin	76	2.43	----------	441	37	63	11	79	13	79	3
Do	Balara filter effluent	272	0.25	----------	85	74	26	0.4	265	2	265	0.4
Do	Balara surge tank	42	0.25	----------	205	48	52	7	110	0	25	4
Do	Balara aërator	40	0.25	----------	416	30	70	10	110	3	25	4
Do	San Juan reservoir	61	0.22	----------	484	52	48	13	68	13	58	3
Do	School of Hygiene tap	45	0.23	0.052	9	100	0	0	206	0	1	0
Do	Other city taps	158	0.25	0.101	9	99	1	0	755	0.4	----------	----------
Do	Suburban taps	108	0.58	0.095	13	98	2	0	521	1	1	0
Do	Suburban taps (partially chlorinated).	62	0.22	----------	68	74	26	0	67	2	57	2

August, 1935	Novaliches raw	31	9.08		522	23	27	13	31	13	31	3
Do	Balara sedimentation basin	76	2.22		309	70	30	9	76	9	76	0
Do	Balara filter effluent	350	0.36		75	82	18	0	350	3	350	0.3
Do	Balara surge tank	33	0.38		72	82	18	0	33	0	33	0
Do	Balara aërator	35	0.35		90	77	23	0	36	6	36	0
Do	San Juan reservoir	61	0.64		100	80	20	2	62	5	62	0
Do	School of Hygiene tap	46	0.56	0.032	4	100	0	0	240	0		
Do	Other city taps	165	0.64	0.141	8	98	2	0	825	1		
Do	Suburban taps	129	0.72	0.129	10	97	3	0	650	1		
Do	Suburban taps (partially chlorinated).	62	0.62		21	98	2	0	61	2	61	0
September, 1935	Novaliches raw	29	5.26		406	7	93	10	29	3	29	0
Do	Balara sedimentation basin	57	2.39		190	35	65	2	57	2	57	2
Do	Balara filter effluent	329	0.57		65	88	12	0	329	0	329	0
Do	Balara surge tank	29	0.58		44	97	3	0	29	0	29	0
Do	Balara aërator	27	0.58		54	96	4	0	27	0	27	0
Do	San Juan reservoir	59	1.23		69	78	22	0	59	2	59	2
Do	School of Hygiene tap	34	1.25	0.020	3	100	0	0	170	0		
Do	Other city taps	155	1.24	0.111	4	100	0	0	775	0		
Do	Suburban taps	128	1.24	0.133	6	99	1	0	640	0		
Do	Suburban taps (partially chlorinated).	63	1.23		12	100	0	0	79	4	59	0
October, 1935	Novaliches raw	31	4.12		120	39	61	0	31	3	31	0
Do	Balara sedimentation basin	66	1.87		83	74	26	0	66	0	66	0
Do	Balara filter effluent	317	0.56		59	93	7	0	317	0	317	0.3
Do	Balara surge tank	31	0.55		46	97	3	0	31	0	31	0
Do	Balara aërator	31	0.55		41	100	0	0	31	0	31	0
Do	San Juan reservoir	62	1.26		21	100	0	0	62	0	62	0
Do	School of Hygiene tap	21	1.29	0.007	2	100	0	0	105	0	0	
Do	Other city taps	175	1.26	0.096	4	99.4	0.6	0	875	0	0	
Do	Suburban taps	132	1.25	0.107	8	98	2	0	660	0.7	0	
Do	Suburban taps (partially chlorinated).	62	1.26		8	100	0	0	62	0	62	0

TABLE 1.—*Monthly records of laboratory examinations of the Metropolitan Water District water from November, 1934, to October, 1935, showing the effects of filtration*—Continued.

SUMMARY.

Date.	Location.	Samples.	Average turbidity.	Average residual chlorine.	Colonies per cc after 24 hours at 37° C.				Test for *coli-aërogenes* group.			
									10-cc portion.		1-cc portion.	
					Mean.	<100.	>100.	>1,000.	Number.	Positive.	Number.	Positive.
			p. p. m.	*p. p. m.*		*P. ct.*	*P. ct.*	*P. ct.*		*P. ct.*		*P. ct.*
No filtration	Montalban raw	83			130	64	36	1	285	41	169	4
Do	Novaliches raw	93	2.83		80	83	17	1	125	10	74	1
Do	Balara raw	106			224	67	33	4	404	34	21	0
Do	San Juan reservoir	220			216	62	38	1	516	38	194	8
Do	School of Hygiene tap	265	2.38	0.075	62	84	16	0.4	1245	3	615	0.2
Do	Other city taps	609	0.17	0.259	42	86	14	0	2548	1		
Do	Suburban taps	284	0.00	0.074	110	69	31	0.7	1066	5		
Experimental filtration	Novaliches raw	91	1.40		235	69	31	3	109	8	86	1
Do	Balara sedimentation basin	186	0.56		75	85	15	1	191	9	177	0.6
Do	Balara filter effluent	300			86	67	33	0.3	372	12	352	3
Do	Balara surge tank	71	0.00		80	79	21	0	346	10	1	0
Do	Balara aërator	78	0.00		172	52	48	0.4	355	28	44	89
Do	San Juan reservoir	164			84	75	25	0	435	38	149	23
Do	School of Hygiene tap	105	0.09	0.060	34	89	11	0	521	0.4	136	0
Do	Other city taps	335	0.05	0.148	15	97	3	0	1665	0.7		
Do	Suburban taps	156	0.08	0.046	56	79	21	0	776	4		
Effective filtration	Novaliches raw	129	6.16		336	22	78	7	131	14	131	6
Do	Balara sedimentation basin	275	2.20		266	55	45	6	278	6	278	1
Do	Balara filter effluent	1268	0.47		71	85	15	0.1	1261	1	1261	0.2
Do	Balara surge tank	135	0.42		101	78	22	2	203	0	118	0.8
Do	Balara aërator	134	0.41		168	72	28	3	204	2	119	0.8
Do	San Juan reservoir	243	0.86		169	78	22	4	251	5	241	1

Do	School of Hygiene tap	146	0.83	0.031	5	100	0	0	721	0	1	0
Do	Other city taps	663	0.94	0.112	6	99	1	0	3230	0.2	0	
Do	Suburban taps	497	1.01	0.117	9	98	2	0	2471	0.6	1	0
Do	Suburban taps (partially chlorinated).	249	0.84		27	93	7	0	269	2	239	0.4

TABLE 2.—*Summary of laboratory examinations of the unfiltered Metropolitan Water District water from September, 1931, to November, 1934.*

Month.	Source.	Samples.	Average turbidity.	Average residual chlorine.	Colonies per cc after 24 hours at 37° C.				Test for *coli-aërogenes* group.					
									10-cc portion.			1-cc portion.		
					Mean.	<100.	>100.	>1,000.	Number.	Positive.	Doubtful.	Number.	Positive.	Doubtful.
			p. p. m.	*p. p. m.*		*P. ct.*	*P. ct.*	*P. ct.*		*P. ct.*	*P. ct.*		*P. ct.*	*P. ct.*
January	Novaliches raw	2	5.00		36	100	0	0	2	0	50	2	50	0
Do	School of Hygiene tap	20	0.77	0.006	5	100	0	0	20	0	15	20	0	5
February	Novaliches raw	2	5.00		2	100	0	0	2	0	0	2	0	0
Do	School of Hygiene tap	17	0.00	0.013	3	100	0	0	17	0	6	17	0	6
March	Novaliches raw	1	20.00											
Do	School of Hygiene tap	18	0.00	0.009	5	100	0	0	17	0	0	17	0	0
April	Novaliches raw	1	5.00											
Do	School of Hygiene tap	16	0.00	0.012	17	100	0	0	13	8	15	15	20	7
May	Novaliches raw	3	1.67		53	100	0	0	1	0	0	1	0	0
Do	School of Hygiene tap	22	0.00	0.007	26	95	5	0	20	35	5	20	5	20
June	Novaliches raw	1	5.00		96	100	0	0	1	0	0	1	0	0
Do	School of Hygiene tap	20	0.00	0.004	25	100	0	0	19	32	5	18	6	6
July	Novaliches raw	1	5.00		57	100	0	0	1	0	100	1	0	100
Do	School of Hygiene tap	21	0.48	0.005	13	95	5	0	21	5	14	13	0	8
August	Novaliches raw	2	5.00		8	100	0	0	1	0	100	1	0	0
Do	School of Hygiene tap	28	0.00	0.010	13	100	0	0	27	11	7	15	0	0

TABLE 2.—*Summary of laboratory examinations of the unfiltered Metropolitan Water District water from September, 1931, to November, 1934*—Continued.

Month.	Source.	Samples.	Average turbidity.	Average residual chlorine.	Colonies per cc after 24 hours at 37° C.				Test for *coli-aërogenes* group.					
									10-cc portion.			1-cc portion.		
					Mean.	<100.	>100.	>1,000.	Number.	Positive.	Doubtful.	Number.	Positive.	Doubtful.
			p. p. m.	*p. p. m.*		*P. ct.*	*P. ct.*	*P. ct.*		*P. ct.*	*P. ct.*		*P. ct.*	*P. ct.*
September	Novaliches raw	4	5.00											
Do	School of Hygiene tap.	31	2.40	0.007	9	100	0	0	28	0	0	12	0	0
October	Novaliches raw	5	5.00		76	50	50	0	4	75	25	4	50	0
Do	School of Hygiene tap.	52	2.30	0.011	11	100	0	0	50	2	20	24	0	8
November	Novaliches raw	3	3.33		19	100	0	0	3	67	33	3	0	33
Do	School of Hygiene tap.	31	1.36	0.012	5	100	0	0	31	10	19	20	0	15
December	Novaliches raw	1	5.00		34	100	0	0	1	100	0	1	100	0
Do	School of Hygiene tap.	16	0.00	0.004	10	94	6	0	16	12	19	16	12	12
Summary	Novaliches raw	26	5.00		43	88	12	0	16	37	83	16	25	12
Do	School of Hygiene tap.	292	0.84	0.009	12	99	1	0	279	9	11	207	3	8

ILLUSTRATIONS

TEXT FIGURES

FIG. 1. Average number of bacteria per cubic centimeter in samples collected from different parts of the Metropolitan water system.
2. Percentage positive for *coli-aërogenes* tests of samples collected from different parts of the Metropolitan water system.

PHYSICOCHEMICAL FACTORS IN ANOPHELINE ECOLOGY, I: STUDIES ON NITROGEN

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INTRODUCTION

That the physical characters of water influence mosquito breeding has been demonstrated by a large number of investigators, among whom were Leicester,(1) Daniels,(2) Watson,(3) Hacker,(4, 5) Lamborn,(6) Iyengar,(7) Senior-White,(8, 9) MacGregor,(10, 11) Barber and Komp,(12) Purdy,(13) Boyd,(14) and Morin and Bader.(15, 16) These investigators have made precise measurements of the physical characteristics of mosquito breeding places, whereas previously such observations were made only through the naked eye, which naturally lacked the desirable accuracy. They also contributed important data towards the advancement of biological control of malaria.

Of the authors mentioned above I wish to cite especially the following: Watson, in his comprehensive publication on malaria prevention,(3) pointed out the importance and need of the study of the physical properties of mosquito breeding places and substantiated his discussions by observations in the field. Lamborn's study in Malaya(6) gives a good summary of the work on the subject of physical factors in mosquito ecology to the end of 1921. His experiments, though incomplete, attracted much attention to the subject. Senior-White(8) summarized the work published to the end of 1925, and made an intensive study based on seven hundred observations. He was able to define the optimum limits of larva preference to pH range and solute concentration, but was unable to show a sharply limiting factor for saline ammonia and dissolved oxygen. Furthermore, he confirmed the "Association-Limit" hypothesis of Hacker. Finally, he observed the feeding association of certain mosquitoes, especially anophelines, with certain algæ.

In his second paper on the subject Senior-White(9) observed that various investigators recorded wide natural ranges of pH

for a number of anopheline species, and that an abundance of larvæ had been found at widely differing extremes. He concluded that pH alone was not a cause appreciably and directly affecting anopheles larvæ within their ordinary range of variation and could not explain mosquito breeding preference. Barber and Komp,(12) working in Alabama, came to the same conclusions.

Lamborn(6) noted that certain types of female mosquitoes were influenced in their choice of breeding place by odors characteristic of various spots. These odors might be those of certain rotting leaves, fetid rice fragments, malodorous sewage, and other odors, which could not be detected by the human power of smell. He showed that the females of certain species were attracted by the presence of certain kinds of plankton.

Purdy,(13) in an investigation of California rice fields, observed the practical absence of mosquito breeding in one particular rice field, when the surrounding collections of water and other rice fields showed breeding. Comparison of this rice field with the others did not indicate any essential difference in number of larval enemies, relative amount of food supply, temperature, alkalinity of water, free carbon dioxide (CO_2), dissolved oxygen, or relative stability. On the other hand, a heavy growth of a blue-green alga (*Tolypothrix*) in this rice field, found only in rare instances and in minute quantities in the others, seemed to be the factor inhibiting mosquito breeding.

Boyd,(14) working on the physical and chemical factors in relation to the distribution of larvæ in northeastern North Carolina, found that the temperature, light, dissolved oxygen, and carbon dioxide were important factors in determining the distribution of the breeding places of anopheles. He observed that the breeding of *Anopheles quadrimaculatus* and *A. crucians* did not become widespread until available places had a mean temperature of 21° C. or higher, and declined as the temperature went below this limit in the fall. He could find no breeding in waters devoid of dissolved oxygen. *Anopheles quadrimaculatus* appeared to favor water with a fairly high concentration of carbon dioxide. On the other hand, *A. punctipennis* was found in waters having as wide a range in the amount of this gas in solution, but apparently did not show the same degree of predilection for higher concentrations as did *A. quadrimaculatus*.

The literature above cited is by no means a complete enumeration of the studies made on physical factors, as it is realized

that many more investigations have been conducted on this subject.

On the chemical composition of mosquito breeding places, however, very few investigations have been reported. Among these, it is interesting to note that Waddell,(17) in 1903, discovered that minute amounts of ammonia were fatal to mosquito larvæ. He observed that a solution of 1 in 4,000 of liquor ammonia proved fatal to mature larvæ and computed that the presence in water of such minute quantities as 1 in 20,000 or 30,000 would be prohibitive to the life of the newly hatched. His experiments indicated that ammonia is poisonous to mosquito larvæ in all its combinations, the nitrogen unit being the index of effectiveness. Unfortunately, this original finding of Waddell had been overlooked for many years until it was rediscovered by Senior-White in 1927.(18)

Lamborn,(6) through the help of Mr. R. W. Blair, chemist, made preliminary analyses of samples of water collected from a swamp and a pond where *A. aconitus* was absent. Lamborn's study tends to indicate that amounts of less than 1 part per million of albuminoid ammonia were inhibitory to the breeding of *A. aconitus,* but his experiments were not continued far enough to warrant definite conclusions.

Senior-White,(8) in his first study on physicochemical factors affecting mosquito breeding, attempted to determine the ammonia-toleration limits of the different types of anopheles in India, but was not successful, due to the difficulties encountered with the use of the direct Nesslerization method for detecting ammonia. However, in a second paper(9) he reported that albuminoid ammonia had no effect on mosquito breeding, but noted that saline ammonia was inhibitory to anopheline breeding, except in the case of the *rossi* group, in amounts exceeding 1 part per million. He remarked that the increase in saline ammonia resulted when saprophytic organisms, engaged in reducing protein substances to ammonia, were acting at a greater rate than the nitrifying organisms. He concluded that the inhibitory effect of small amounts of ammonia to breeding points to the possibility of a practical biological control by the employment of a bacteriophage to inhibit the growth of *Nitrosomonas,* thereby raising the ammonia content of waters to a lethal figure. On the other hand, Waddell(17) would effect the increase of ammonia by the use of leguminous plants in agricultural rotations.

Williamson,(19) in an extensive study of the subject, showed that correlations existed between certain organic factors and anopheles breeding. Williamson showed that each species had its range of tolerance, though the limit was not determined definitely. His paper also included a summary of previous studies on the effect of mineral salts on anopheles breeding. According to him these studies tend to show that certain mineral factors, as varying concentration of chlorides, phosphates, magnesium, and iron, exerted a certain influence, though not exactly a definable one, except in the case of salinity and iron content, upon the breeding of certain species of mosquitoes. He confirmed the findings of previous workers that iron salts in high concentrations were detrimental to anopheles larvæ.

Williamson,(20) in another publication, explained that the chemical factors detrimental to mosquito larvæ probably operate largely through determining the quality of the food, as this is modified by antecedent occurrences in the nitrogen cycle. He pointed to the possibility that the ratio between the oxidized and ammoniacal nitrogen, which he called nitrification ratio, is responsible for the inhibitory effect upon the anopheline larvæ. The inhibiting effect is obtained when the ratio is less than one. He suggested that consideration of ratios of a group of important causal factors may be more important than taking each of them singly.

Rudolfs and Lackey,(21) in a study of aëdes and culex mosquitoes, made an extensive examination, lasting for several years, for the pH, carbon dioxide, HCO_3, carbonate, chloride, sulphate, free ammonia, albuminoid ammonia, total nitrogen, carbon, aluminium, and iron in two pools: One where supposedly no breeding would take place, and the other a typical woodland pool where continuous breeding of *Aëdes canadensis* occurred. The authors concluded that *A. canadensis* was present in the pool with either high or low total acidity, with free carbon dioxide of 4 and 40 parts per million with 5 and 75 parts per million chlorides, with 2 and 112 parts per million sulphates, with high and low acid carbonates, free ammonia, and albuminoid ammonia. Their finding showed that the carbon-nitrogen ratios of the water did not seem to affect breeding. Larvæ of *C. pipiens* were killed when the reaction of natural water was changed gradually.

In another publication Senior-White(18) reported that the pH, conductivity, carbonates, and albuminoid ammonia were useless

in connection with mosquito breeding. However, he reserved his opinion on the "residual pH," phosphates, and dissolved oxygen, which need further study. He reported that saline ammonia was inhibitory to anopheline breeding, except in the case of the *rossi* group, in amounts exceeding 1 part per million.

From the review of the literature on the subject it seems that explanations and conclusions on the influence of chemical factors on mosquito ecology are still speculative, due to the meagerness of the work done so far. Therefore, it seems that further investigation and research are needed to throw more light on this important subject.

PROCEDURE OF INVESTIGATION

Our investigation deals principally with the examination of some physical and chemical factors affecting the breeding of *Anopheles minimus* var. *flavirostris* and the associated moving-water group of anopheles. The purpose of my present paper is first to determine the natural composition of typical breeding places of *A. minimus*. Later I hope to find the breeding preference of this mosquito for different types of water, and whether or not the varying composition of waters has any influence upon its breeding. If possible I intend to determine the approximate toleration-limit of *A. minimus* to the various chemical constituents of water. *Anopheles minimus* was chosen for this study because it was found by Manalang(22) to be the most efficient vector of malaria in the Philippines.

The first part of my study was confined mostly to Bayan Creek, a typical *minimus* breeding place in the town of San Jose (100 meters' elevation), Bulacan Province, Luzon. This creek is small, sluggish, and tortuous, and changes markedly its cross section and speed at frequent intervals of its course. During the dry months of April and May it has a mean discharge of 0.15 cubic feet per second, but during the rainy months, from July to October, its mean discharge increases to 15 cubic feet or more per second. Its average width at points of observation is from 5 to 7 feet during the dry months, but this is increased to about 7 to 11 feet during heavy rains. It has a mean depth of 0.5 foot during the dry months and 2 feet during the rainy season. My previous study(23) showed that *Anopheles minimus* larvæ were found in portions of the creek with a velocity range, 1 foot from the shore, of from 0.05 to 0.55 foot per second.

Bayan Creek was covered by a variety of vegetation along the edges, notable among which was a species of grass (*Paspalum conjugatum* Berg.) whose leaves overhung the water and afforded good shelter for larvæ. The débris along the creek consisted of roots, twigs, and decaying leaves of bamboo and various trees and shrubs.

In many parts of the creek the banks were steep, and in some places overhanging. *Anopheles minimus* was found in abundance along the edges of these banks, especially where a small amount of shade was afforded by bamboo or other trees. In most places sand and gravel were found at the bottom of the creek, but in those portions frequented by carabaos a thin layer of mud was found at the bottom. No control measure was used on the creek during our experiments.

Besides the samples from Bayan Creek a few samples of water were also collected from other *minimus* breeding places in Los Baños and Calauan, Laguna Province, and in Anabo, Cavite Province. In the latter place stagnant pools by the sides of the stream were examined because they were occasionally found positive for *A. minimus* larvæ.

A number of stations along the breeding place were selected for observation. In each station samples of water were taken, the description was recorded, and the temperature of the water noted. The collections for larvæ were made on an average of five dips, with a dipper holding 800 cubic centimeters. All larvæ collected were counted and identified.

The samples of water were examined immediately in the laboratory in Manila, but when they could not be examined upon arrival they were placed in a refrigerator until the next day. For the purpose of this study I included the examinations for turbidity, ammonia and albuminoid nitrogen, nitrite and nitrate nitrogen, chloride, total iron, dissolved oxygen, and carbon dioxide. Observations from the same station were made weekly during the first three months, later at an average of once every two weeks.

In the analyses for the ammonia and albuminoid nitrogen the distillation method was employed, following the procedure recommended in the Standard Methods of Water Analysis of the American Public Health Association.(24) My analyses for turbidity, nitrite, nitrate, chloride, and total iron conform to the same Standard Methods and all results were recorded in parts per million. Only chemically pure chemicals obtained from re-

liable drug manufacturers abroad were utilized in the analyses. Solutions of these chemicals were renewed from time to time. Before the analyses were started, the samples were filtered in cotton to remove large suspended objects, as leaves, débris, larvæ, etc.

RESULTS AND DISCUSSION

For the sake of clearness I am reporting the results of the present study in several papers. Only the findings on the nitrogen content of natural breeding places of *Anopheles minimus* and associated anopheles are included in this report. The results on chlorides, iron, and other physicochemical factors related to anopheles breeding will be treated in succeeding papers.

A summary of the results of analyses of about 170 samples of water for different forms of nitrogen is found in Tables 1 to 6. Table 1 shows the occurrence and intensity of anopheline breeding in different concentrations of ammonia nitrogen. It will be seen that most of the observations for *A. minimus* occur on breeding places with concentrations of 0.025 or below to 0.124 part per million of ammonia nitrogen and the greatest abundance of larvæ was found within these concentrations.

In Table 2 it will be noted that *A. minimus* occurred mostly in concentrations of 0.050 to 0.349 part per million of albuminoid nitrogen. In Table 3 it will be observed that most of the samples were between concentrations of 0.0 to 0.0079 part per million of nitrite nitrogen, and in Table 4 between 0.010 and 0.049 part per million of nitrate nitrogen. These figures give the natural range of saline concentration at various points during different times. They do not necessarily mean toleration limits for *A. minimus,* inasmuch as even in the maximum concentrations encountered in this study, *A. minimus* was found breeding.

The four tables show that *A. barbirostris* was the most constant associate of *A. minimus,* in Bayan Creek at least. The tables also give the occurrence and abundance of *A. barbirostris* and other anopheles commonly associated with *A. minimus* in relation to different concentrations of nitrogenous substances.

Table 5 records the mean concentrations of different forms of nitrogen in typical *A. minimus* breeding places of Bulacan, Laguna, and Cavite in different months and years included in this study. Table 6 is a summary of Table 5. These tables show that natural breeding places of *A. minimus* give low concentrations of nitrogen, characteristic of those found in unpolluted natural surface waters. An exception is found in the

TABLE 1.—*Occurrence and intensity of anopheline breeding in different concentrations of ammonia nitrogen.*

Ammonia nitrogen in part per million.	*Anopheles minimus.*			*Anopheles barbirostris.*			*Anopheles aconitus.*		
	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.
	Total.	Positive.		Total.	Positive.		Total.	Positive.	
		P. ct.			*P. ct.*			*P. ct.*	
0.000–0.024	14	50	1.26	14	64	1.56	14	29	0.20
0.025–0.049	28	79	1.12	28	61	1.27	28	14	0.03
0.050–0.074	20	90	1.99	20	80	1.20	20	10	0.09
0.075–0.099	12	92	0.77	12	82	1.27	12	9	0.02
0.100–0.124	6	67	1.33	6	67	0.33	6	33	0.07
0.125–0.149	3	33	0.13	3	100	0.67	3	0	0.00
0.150–0.174	2	100	0.50	2	100	0.60	2	0	0.00
0.175–0.199	1	100	0.30	1	100	0.20	1	0	0.00
0.200–0.224	1	100	0.60	1	100	1.00	1	0	0.00
0.225–0.249	2	100	4.50	2	50	0.10	2	0	0.00
0.250–0.274									
0.275–0.299	1	100	10.00	1	100	0.60	1	0	0.00
0.300–0.324	2	50	1.50	2	50	0.40	2	0	0.00
0.325–0.349									
0.350–0.374	2	100	0.50	2	50	0.30	2	50	0.10

Ammonia nitrogen in part per million.	*Anopheles vagus.*			*Anopheles fuliginosus.*			Unidentified.			All species.		
	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.
	Total.	Positive.		Total.	Positive.		Total.	Positive.		Total.	Positive.	
		P. ct.			*P. ct.*			*P. ct.*			*P. ct.*	
0.000–0.024	14	21	0.06	14	14	0.09	14	57	0.76	14	79	3.93
0.025–0.049	28	0	0.00	28	4	0.01	28	64	0.50	28	89	2.93
0.050–0.074	20	15	0.05	20	0	0.00	20	75	1.05	20	95	4.38
0.075–0.099	12	9	0.13	12	0	0.00	12	64	1.21	12	100	3.40
0.100–0.124	6	0	0.00	6	0	0.00	6	50	0.73	6	67	2.46
0.125–0.149	3	0	0.00	3	0	0.00	3	67	0.93	3	100	1.73
0.150–0.174	2	0	0.00	2	0	0.00	2	100	0.60	2	100	1.70
0.175–0.199	1	0	0.00	1	0	0.00	1	0	0.00	1	100	0.50
0.200–0.224	1	0	0.00	1	0	0.00	1	100	0.40	1	100	2.00
0.225–0.249	2	0	0.00	2	0	0.00	2	100	1.40	2	100	6.00
0.250–0.274												
0.275–0.299	1	0	0.00	1	0	0.00	1	100	10.80	1	100	21.40
0.300–0.324	2	0	0.00	2	0	0.00	2	50	0.50	2	100	2.40
0.325–0.349												
0.350–0.374	2	0	0.00	2	0	0.00	2	50	2.80	2	100	3.70

TABLE 2.—*Occurrence and intensity of anopheline breeding in different concentrations of albuminoid nitrogen.*

Albuminoid nitrogen in part per million.	*Anopheles minimus.*			*Anopheles barbirostris.*			*Anopheles aconitus.*		
	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.
	Total.	Positive.		Total.	Positive.		Total.	Positive.	
		P. ct.			*P. ct.*			*P. ct.*	
0.050–0.099	11	45	0.80	11	45	0.80	11	9	0.02
0.100–0.149	19	84	1.20	19	90	1.74	19	37	0.24
0.150–0.199	22	77	1.77	22	64	1.44	22	9	0.03
0.200–0.249	14	100	2.87	14	79	0.97	14	14	0.03
0.250–0.299	18	72	0.80	18	50	0.64	18	6	0.01
0.300–0.349	6	83	1.40	6	50	0.20	6	0	0.00
0.350–0.399	1	100	1.60	1	100	0.20	1	0	0.00
0.400–0.449									
0.450–0.499	1	0	0.00	1	100	0.40	1	0	0.00
0.500–0.549	1	100	0.40	1	100	1.40	1	0	0.00
0.550–0.599	2	100	1.00	2	100	1.80	2	0	0.00
0.600–0.699	1	0	0.00	1	0	0.00	1	0	0.00
0.700–0.799	2	0	0.00	2	50	0.80	2	0	0.00
0.800–0.899	1	100	0.60	1	100	0.60	1	100	0.20
0.900–0.999	1	0	0.00	1	0	0.00	1	0	0.00

Albuminoid nitrogen in part per million.	*Anopheles vagus.*			*Anopheles fuliginosus.*			Unidentified.			All species.		
	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.
	Total.	Positive.		Total.	Positive.		Total.	Positive.		Total.	Positive.	
		P. ct.			*P. ct.*			*P. ct.*			*P. ct.*	
0.050–0.099	11	18	0.04	11	0	0.00	11	36	0.54	11	55	2.20
0.100–0.149	19	16	0.12	19	0	0.00	19	74	0.71	19	100	4.01
0.150–0.199	22	5	0.01	22	9	0.03	22	82	1.56	22	95	4.84
0.200–0.249	14	7	0.04	14	7	0.06	14	86	1.67	14	100	5.64
0.250–0.299	18	0	0.00	18	0	0.00	18	39	0.38	18	83	1.83
0.300–0.349	6	17	0.03	6	0	0.00	6	50	0.97	6	100	2.60
0.350–0.399	1	0	0.00	1	0	0.00	1	100	0.80	1	100	2.60
0.400–0.449												
0.450–0.499	1	0	0.00	1	0	0.00	1	100	0.60	1	100	1.00
0.500–0.549	1	0	0.00	1	0	0.00	1	100	2.20	1	100	4.00
0.550–0.599	2	0	0.00	2	0	0.00	2	100	1.20	2	100	4.00
0.600–0.699	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00
0.700–0.799	2	0	0.00	2	0	0.00	2	0	0.00	2	50	0.80
0.800–0.899	1	0	0.00	1	0	0.00	1	0	0.00	1	100	1.40
0.900–0.999	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00

TABLE 3.—*Occurrence and intensity of anopheline breeding in different concentrations of nitrite nitrogen.*

Nitrite nitrogen in part per million.	*Anopheles minimus.*			*Anopheles barbirostris.*			*Anopheles aconitus.*		
	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.
	Total.	Positive.		Total.	Positive.		Total.	Positive.	
		P. ct.			*P. ct.*			*P. ct.*	
0.0000–0.0019	20	60	1.41	20	65	1.54	20	15	0.05
0.0020–0.0039	17	82	1.23	17	71	1.23	17	18	0.12
0.0040–0.0059	7	100	2.49	7	57	0.54	7	29	0.29
0.0060–0.0079	5	100	1.00	5	80	0.84	5	40	0.08
0.0080–0.0099	4	75	1.10	4	25	0.20	4	0	0.00
0.0100–0.0149	3	100	0.33	3	0	0.00	3	0	0.00
0.0150–0.0199	2	50	1.50	2	0	0.00	2	0	0.00
0.0200–0.0249	3	67	4.27	3	33	0.13	3	0	0.00
0.0250–0.0299	1	100	3.00	1	0	0.00	1	0	0.00
0.0300–0.0399	1	100	0.80	1	0	0.00	1	0	0.00
0.0400–0.0499	1	100	0.20	1	0	0.00	1	0	0.00
0.0500–0.0999	1	100	1.60	1	100	0.20	1	0	0.00
0.1000–0.1499	1	100	5.20	1	0	0.00	1	0	0.00
0.1500–0.1999									
0.2000–0.2999	2	100	7.00	2	100	0.50	2	50	0.10

Nitrite nitrogen in part per million.	*Anopheles vagus.*			*Anopheles fuliginosus.*			Unidentified.			All species.		
	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.
	Total.	Positive.		Total.	Positive.		Total.	Positive.		Total.	Positive.	
		P. ct.			*P. ct.*			*P. ct.*			*P. ct.*	
0.0000–0.0019	20	5	0.03	20	5	0.01	20	45	0.76	20	75	3.80
0.0020–0.0039	17	12	0.04	17	0	0.00	17	65	0.71	17	88	3.33
0.0040–0.0059	7	29	0.06	7	0	0.00	7	86	1.06	7	100	4.44
0.0060–0.0079	5	0	0.00	5	0	0.00	5	80	0.96	5	100	2.88
0.0080–0.0099	4	0	0.00	4	0	0.00	4	25	1.50	4	75	2.80
0.0100–0.0149	3	0	0.00	3	0	0.00	3	33	1.87	3	100	2.20
0.0150–0.0199	2	0	0.00	2	0	0.00	2	50	0.50	2	50	2.00
0.0200–0.0249	3	33	0.07	3	0	0.00	3	100	1.10	3	100	5.57
0.0250–0.0299	1	0	0.00	1	0	0.00	1	100	1.00	1	100	4.00
0.0300–0.0399	1	0	0.00	1	0	0.00	1	100	0.40	1	100	1.20
0.0400–0.0499	1	100	1.60	1	0	0.00	1	100	1.00	1	100	2.80
0.0500–0.0999	1	0	0.00	1	0	0.00	1	100	0.80	1	100	2.60
0.1000–0.1499	1	100	0.20	1	0	0.00	1	100	5.00	1	100	10.40
0.1500–0.1999												
0.2000–0.2999	2	0	0.00	2	0	0.00	2	100	7.10	2	100	14.70

TABLE 4.—*Occurrence and intensity of anopheline breeding in different concentrations of nitrate nitrogen.*

Nitrate nitrogen in part per million.	*Anopheles minimus.*			*Anopheles barbirostris.*			*Anopheles aconitus.*		
	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.
	Total.	Positive.		Total.	Positive.		Total.	Positive.	
		P. ct.			*P. ct.*			*P. ct.*	
0.000–0.009	3	67	1.40	3	100	2.60	3	0	0.00
0.010–0.019	12	100	2.17	12	83	2.03	12	42	0.32
0.020–0.029	11	91	1.35	11	82	1.35	11	45	0.15
0.030–0.039	11	36	0.33	11	36	0.45	11	0	0.00
0.040–0.049	11	91	1.46	11	27	0.45	11	0	0.00
0.050–0.059	2	50	1.40	2	50	0.20	2	0	0.00
0.060–0.069	3	100	6.60	3	67	0.53	3	0	0.00
0.070–0.079	3	100	3.80	3	33	0.13	3	33	0.07
0.080–0.089	2	100	0.20	2	50	0.10	2	0	0.00
0.090–0.099	1	100	3.00	1	0	0.00	1	0	0.00
0.100–0.124	4	50	1.80	4	50	0.40	4	0	0.00
0.125–0.149	1	0	0.00	1	0	0.00	1	0	0.00
0.150–0.199	1	100	1.60	1	100	0.20	1	0	0.00
0.200–0.299	1	100	1.00	1	100	0.80	1	0	0.00
0.300–0.399	2	100	2.80	2	0	0.00	2	0	0.00

Nitrate nitrogen in part per million.	*Anopheles vagus.*			*Anopheles fuliginosus.*			Unidentified.			All species.		
	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.	Observations.		Average larvæ per dip.
	Total.	Positive.		Total.	Positive.		Total.	Positive.		Total.	Positive.	
		P. ct.			*P. ct.*			*P. ct.*			*P. ct.*	
0.000–0.009	3	0	0.00	3	0	0.00	3	0	0.00	3	100	4.00
0.010–0.019	12	17	0.05	12	8	0.02	12	83	0.83	12	100	5.42
0.020–0.029	11	27	0.09	11	0	0.00	11	73	1.36	11	100	4.30
0.030–0.039	11	0	0.00	11	0	0.00	11	27	0.22	11	45	1.00
0.040–0.049	11	0	0.00	11	0	0.00	11	64	1.66	11	91	3.57
0.050–0.059	2	0	0.00	2	0	0.00	2	100	0.85	2	100	2.45
0.060–0.069	3	0	0.00	3	0	0.00	3	100	4.53	3	100	11.66
0.070–0.079	3	0	0.00	3	0	0.00	3	100	2.07	3	100	6.07
0.080–0.089	2	0	0.00	2	0	0.00	2	50	0.10	2	100	0.40
0.090–0.099	1	0	0.00	1	0	0.00	1	100	1.00	1	100	4.00
0.100–0.124	4	25	0.05	4	0	0.00	4	25	1.25	4	75	3.50
0.125–0.149	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00
0.150–0.199	1	0	0.00	1	0	0.00	1	100	0.80	1	100	2.60
0.200–0.299	1	100	0.00	1	0	0.00	1	100	1.60	1	100	3.40
0.300–0.399	2	100	0.90	2	0	0.00	2	100	1.00	2	100	4.70

TABLE 5.—*Mean concentrations of ammonia nitrogen, albuminoid nitrogen, nitrite nitrogen, and nitrate nitrogen in Anopheles minimus breeding places, classified by month and year.*

Place, month, and year.	Observations.		Average larvæ per dip.	Temperature.	Salt concentrations in part per million.			
	Total.	Positive.			Ammonia nitrogen.	Albuminoid nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.
BULACAN.		*P. ct.*		°*C.*				
April, 1931	3	100	1.40	32.2	0.225	0.576		
May, 1931	12	67	0.45	32.2	0.114	0.362		
June, 1931	13	54	0.42	30.0	0.071	0.235		
July, 1931	11	36	0.25	30.9	0.078	0.214		
August, 1931	2	0	0.00	26.0	0.020	0.108		
September, 1931	6	0	0.00	27.4	0.071	0.154	0.0007	0.000
October, 1931	3	33	0.40	28.3	0.074	0.161	0.0007	0.000
November, 1931	3	100	3.93	27.7	0.047	0.173	0.0010	0.018
January, 1932	7	86	1.71	24.5	0.038	0.113	0.0024	0.007
February, 1932	4	75	0.60	26.4	0.025	0.103	0.0018	0.018
March, 1932	2	100	3.00	26.4	0.011	0.152	0.0035	0.020
April, 1932	32	100	1.68	27.1	0.166	0.166	0.0378	0.039
May, 1932	3	100	3.13	29.6	0.063	0.255	0.0020	0.030
June, 1932	5	80	0.34	28.3	0.093	0.146	0.0020	0.048
July, 1932	2	0	0.00	29.1	0.035	0.121	0.0020	0.030
August, 1932	4	0	0.00	28.4	0.071	0.097	0.0005	0.020
October, 1932	4	0	0.00	27.9	0.038	0.087	0.0012	0.028
December, 1932	2	0	0.00	24.8	0.030	0.078	0.0010	0.035
May, 1933	2	100	1.70	28.5	0.042	0.267	0.0020	0.035
March, 1934	4	50	1.10	30.1	0.245	0.501	0.0050	0.095
LAGUNA.								
January, 1932	1	0	0.00	26.0	0.092	0.184	0.0200	0.050
March, 1932	4	100	4.90	29.2	0.132	0.149	0.1400	0.195
May, 1932	2	100	0.90	28.2	0.060	0.303	0.0075	0.055
February, 1933	5	80	2.88	24.3	0.116	0.191	0.0100	0.078
March, 1933	6	80	4.20	26.4	0.465	0.214	0.0333	0.058
June, 1933	7	80	0.84	25.5	1.166	0.446	0.0140	0.043
January, 1934	2	100			0.100	0.432	0.0070	0.065
February, 1934	[a] 3	0	0.00	22.6	0.295	0.630	0.0023	0.060
April, 1934	3	67	0.40	20.5	0.063	0.142	0.0060	0.063
June, 1934	3	33	0.20	25.1	0.061	0.207	0.0013	0.033
July, 1934	2	100	1.30	27.5	0.148	0.230	0.0295	0.175
CAVITE.								
February, 1932	8	75	0.52	26.0	0.031	0.326	0.0059	0.034
April, 1932	1	100	0.60	31.4	0.034	0.230	0.0020	0.010
Do	[b] 2	100	1.55	33.2	1.076	0.883	0.0030	0.020

[a] Heavy rain and floods. [b] Stagnant pools.

case of two stagnant pools in Cavite Province where the concentration of ammonia and albuminoid nitrogen was much higher than that found in streams. However, more data are needed to verify this finding.

The records for August and September were negative for larvæ, due to heavy rains and floods which washed them away.

TABLE 6.—*Mean concentrations of ammonia nitrogen, albuminoid nitrogen, nitrite nitrogen, and nitrate nitrogen in Anopheles minimus breeding places, classified by month.*

Month.	Observations.		Average larvæ per dip.	Temperature.	Salt concentrations in part per million.			
	Total.	Positive.			Ammonia nitrogen.	Albuminoid nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.
		P. ct.		°C.				
January	10	80	1.50	24.7	0.056	0.184	0.0051	0.023
February	20	65	1.05	25.0	0.089	0.299	0.0053	0.044
March	16	80	3.40	27.6	0.270	0.262	0.0491	0.097
April	41	98	1.25	27.4	0.203	0.222	0.0325	0.039
May	19	79	1.05	28.3	0.091	0.327	0.0036	0.039
June	28	62	0.46	28.3	0.348	0.269	0.0075	0.043
July	15	40	0.36	26.6	0.082	0.203	0.0157	0.102
August	6	0	0.00	27.2	0.054	0.101	0.0005	0.020
September	6	0	0.00	27.4	0.071	0.154	0.0007	0.000
October	7	14	0.17	28.1	0.053	0.119	0.0010	0.013
November	3	100	3.93	27.7	0.047	0.173	0.0010	0.013
December	2	0	0.00	24.8	0.030	0.078	0.0010	0.035

The same condition accounted for the percentage of negative observations made during the other months of the rainy season. The reasons for the negative results in the observations made during dry seasons are perhaps the variations in nitrogen and other physicochemical factors, the changes in the velocity of the stream at different stations, food supply, degree of shade or sun, and other factors.

Due to the small number of breeding places examined, this paper is presented as a preliminary report to give an idea of the composition of a typical *A. minimus* breeding place. In another paper more extensive data will be presented covering a larger number and variety of breeding places. It is hoped that further study may reveal specific differences in the breeding preference of *A. minimus* with a view to utilizing the findings in larval control.

SUMMARY AND CONCLUSION

A typical breeding place of *Anopheles minimus* var. *flavirostris* was examined for its nitrogen content, but for comparison a few examinations were also made of some other breeding places. The results of analyses of about 170 samples of water collected from these breeding places show that *A. minimus* breeds in bodies of water, notably streams, with low concentrations of ammonia nitrogen, albuminoid nitrogen, nitrite nitrogen, and

nitrate nitrogen, characteristic of unpolluted natural surface waters. The results on chlorides, iron, and other chemicophysical factors related to anopheles breeding will be reported in other papers.

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IS VITAMIN B_2 THE ACCELERATING FACTOR IN THE FERMENTATION OF SUGAR BY PROPIONIC ACID ORGANISMS?[1]

By V. G. Lava, R. Ross, and K. C. Blanchard

That the nitrogen source exerts considerable influence on sugar fermentation by propionic acid bacteria has been known for some time. Sherman(1) preferred milk whey to any other substance for his *Bacterium acidi propionici d.;* and Van Niel,(2) working with different strains of propionic acid bacteria, found that yeast extracts and yeast autolysates are better media than "Poulene," "Witte," "Bacto," and "Proteose" peptones; he showed that the differences in fermentation cannot be ascribed to differences in metabolism, buffer capacity, or nitrogen content, and suggested that the vitamin content of the yeast extract might play an important part.

Our experiments show that the factor in yeast extract responsible for increased fermentation is heat-stable in neutral or very acid conditions, is carried with the lead acetate precipitate at pH 7 to 7.5, and gives a progressive potency if purified according to the method of Euler(3) for vitamin B_2 separation.

EXPERIMENTAL

Throughout all our experiments yeast extracts were obtained according to the method of Van Niel with slight modifications: 250 g of Fleishman's compressed yeast was triturated with 1 liter of tap water, autoclaved with one egg white for thirty minutes at 122° C., filtered, and heated in an Arnold sterilizer for thirty minutes. In our case we added 3 cc concentrated hydrochloric acid (HCl) before autoclaving and, after filtering, further clarified the extract by adding another egg white before sterilizing in the Arnold. The extract obtained in this way was a clear yellow liquid.

The organism used was *Bacterium acidi propionici shermanii,* obtained from Doctor Sherman, of Cornell University. This

[1] Read before the Third Philippine Science Convention February 26, 1935. This work was undertaken in the Department of Biology, New York University.

organism was found to behave in the same way as an identical strain kindly sent us by the United States Department of Agriculture. Cultures were prepared of 25 cc of yeast extract containing 2 per cent of chemically pure glucose, inoculated with 1 cc and incubated at 30° C. for one week. Conditions of the highest sterility were always maintained. Unless otherwise stated, all flasks used in our fermentation experiments contained this 2 per cent glucose concentration.

As an index of the extent of fermentation the total acidity in each flask, containing 150 cc solution, was determined. Ducloux determinations were occasionally made to check the constancy of the $\frac{\text{propionic acid}}{\text{acetic acid}}$ ratio, and to determine indirectly whether bacterial contamination occurred. The pH of the medium at the time of inoculation was approximately 7 (range 6.8 to 7.6) ; the pH was determined with the antimony electrode, using the formula for the saturated calomel electrode at room temperature,

$$\text{pH} = \frac{0.005 \div \text{E. M. F.}}{0.058}$$

In order to determine which concentration of yeast extract was most suitable for our needs, fermentation experiments were carried out using various dilutions. Table 1 shows a typical relationship between titration value [in cc 0.1 *N* sodium hydroxide (NaOH)] after fifteen days' incubation and various dilutions of yeast extract. From these values a dilution of 1 : 3—that is, 50 cc yeast extract in 150 cc total volume—was selected for further experiments.

TABLE 1.—*Effect of concentration of yeast extract.*

Concentration of yeast extract.	pH (15 days).	0.1 *N* Sodium hydroxide (NaOH).	$\frac{HP}{HAc.}$
		cc.	
1/1	4.7	142.7	1:1.3
1/5	4.6	25.7	----------
1/25	----------	4.8	----------
1/75	----------	1.1	----------
1/375	----------	0.7	----------

As it was fairly difficult to carry on experiments at the same salt concentration, the effect of salt concentration upon fermen-

tation was next determined. Table 2 shows the relation between salt concentration, calculated in terms of salt (NaCl) percentage, and titration value after fifteen days' incubation. In succeeding experiments corrections for salt concentration were made according to data in Table 2.

TABLE 2.—*Effect of salt concentration.*

[50 cc of yeast extract in 150 cc of medium and 2 per cent glucose.]

	Salt (NaCl).	pH (15 days).	Titration value 0.1 *N* sodium hydroxide (NaOH).	Acid produced.
	Per cent.		*cc.*	*Per cent.*
Experiment I	0.0	4.1	47.8	100.0
Do	0.13	4.1	47 8	100.0
Do	0.47	4.1	45.3	94 7
Do	0.93	4.2	43.3	90 4
Do	1.33	4.3	42.1	88.4
Experiment II	0.0	4.2	62.2	100.0
Do	2.0	4.2	46.2	74.3
Do	4.0		0.0	0.0

For experiments in which complete fermentation is necessary, it is important that the time-titration value curve be first determined. Table 3 shows this relation. It is evident from

TABLE 3.—*Relation between titration value and time of fermentation.*

Time (days).	pH after fermentation.	Titration value 0.1 *N* sodium hydroxide (NaOH).
4	4.6	19.8
8	4.0	40.6
13	4.0	60.2
20	3.8	70.5
68		70.5

these data that three weeks are sufficient for maximum fermentation. In all the succeeding experiments recorded, unless otherwise stated, three weeks is the incubation period.

EFFECT OF HEATING YEAST EXTRACTS AT DIFFERENT PH'S ON FERMENTATION

Portions consisting of 50 cc of yeast extract at pH 5.1 were treated with various amounts of hydrochloric acid (HCl) and sodium hydroxide (NaOH), made up to 100 cc and autoclaved

for thirty-five minutes, at 122° C. The pH in each flask was then adjusted to 7.1 and the flasks were heated in an Arnold sterilizer for twenty-five minutes, the pH again adjusted to 7.1, and the solutions made up to 150 cc after the addition of 3 g of glucose. The flasks were given a final sterilization in the Arnold, cooled overnight, and inoculated. Table 4 shows

TABLE 4.—*Effect of hydrolysis of yeast extract of different pH's on the titration value.*

0.2 *N* sodium hydroxide (NaOH) or hydrochloric acid (HCl) added before autoclaving.	pH after autoclaving.	0.2 *N* NaOH or HCl to make to pH 7.1.	pH (15 days).	Titration value cc 0.1 *N* NaOH.	Salt concentration.	Acidity corrected for salt concentration.	HP / HAc.
cc.		*cc.*			*Per cent.*	*Per cent.*	
20.0 (HCl)	2.2	22.5 (NaOH)	4.0	66.3	0.17	118	1:1.7
0.0 (NaOH)	5.1	3.5 (NaOH)	4.0	60.3	0.03	100	
1.0 (NaOH)	5.9	2.4 (NaOH)	4.0	61.5	0.03	109	
2.0 (NaOH)	6.4	1.4 (NaOH)	4.0	66.9	0.03	118	
3.0 (NaOH)	6.7	1.1 (NaOH)	4.0	62.4	0.03	110	
4.0 (NaOH)	7.2	0.1 (NaOH)	4.0	63.3	0.03	112	
5.0 (NaOH)	7.6	0.8 (HCl)	4.0	63.3	0.04	112	
6.0 (NaOH)	8.0	1.5 (HCl)	4.0	62.1	0.04	109	
8.5 (NaOH)	8.6	3.4 (HCl)	4.1	62.1	0.06	109	
45.0 (NaOH)	12.6	37.2 (HCl)	4.3	54.3	0.32	99	
35.3 (NaOH)	13.3	31.5 N(H_2SO_4)	7.0	0.0	1.36	0	

the effect of hydrolysis of yeast extract at different pH's on the titration value. It is evident from these figures that heating at a low pH maintains a high titration value, this value becoming lower near pH 4, increasing again to its maximum at pH 6.5, and gradually decreasing with increasing pH, till near pH 12.6 it decreases suddenly, and finally becomes zero at pH 13.3.

POTENCY OF FRACTIONS FROM LEAD ACETATE PRECIPITATION

Unfortunately data in the literature are conflicting regarding the effect of heat at different pH's on the stability of various water-soluble accessory food factors in yeast, and no very definite conclusions can be drawn from our results above as to what group our fermentation factor belongs in. However, the method that we used for the separation of different vitamin B fractions from yeast extract gives a qualitative idea as to where this factor is found most concentrated. It has been shown(4) that lead acetate at pH 7 precipitates most of the vitamin B_2 from the rest of the water-soluble factors in the filtrate. Treatment of this filtrate with Hopkins's mercuric sulphate ($HgSO_4$) re-

agent precipitates most of vitamins B_1 and B_4, leaving a filtrate that when treated with activated charcoal at pH 7 yields an adsorbate, which on further treatment with dilute hydrochloric acid (HCl) yields the X factor and on subsequent treatment of the charcoal with 50 per cent acid alcohol yields the Y factor.

In our experiments yeast extract was treated with neutral lead acetate, adjusted to pH 7 to 7.5 with sodium hydroxide (NaOH), and filtered. The filtrate was freed of lead by precipitating with sulphuric acid (H_2SO_4) and filtering, and the resulting filtrate was adjusted to 0.1 *N* acidity with saturated barium hydroxide [$Ba(OH)_2$]. This was filtered and yielded solution II. The precipitate of the lead acetate treatment was suspended in water and also treated with sulphuric acid (H_2SO_4) and filtered, and the acidity of the resulting filtrate was adjusted to 0.1 *N* with barium hydroxide [$Ba(OH)_2$] and filtered. The resulting filtrate is solution III. Throughout the processes as little heat as was compatible with the clearness of the solutions was administered.

As in preliminary experiments, we found that magnesium and phosphate ions increased fermentation; these were added in the form of potassium hydrogen phosphate (K_2HPO_4) (0.1 per cent) and magnesium sulphate ($MgSO_4.7H_2O$) (0.05 per cent). These concentrations were found by Pett and Wynne[5] to be the optimum concentrations for maximum fermentation of hexose phosphate and glycero-phosphate esters by dried *Bacterium acidi propionici jensenii* Van Niel. Table 5 shows the fermentation value for yeast extract, lead acetate filtrate, and lead acetate precipitate, separately and when mixed together, with and without Mg or PO_4 ions or with both. All these solutions were first adjusted to pH 7, sterilized, and readjusted to pH 7 before the addition of glucose and final sterilization.

The original total nitrogen content was determined in each solution by the Kjeldahl method to serve as a basis for determining the potency of each fraction. The data show that for the same amount of nitrogen the lead acetate precipitate (solution III) yields a more potent fraction than the lead acetate filtrate (solution II) and that the original yeast extract gave fermentation values between these two.

The difference in fermentation value cannot be due to differences in physiological activity of the organism nor to the general method of procedure, because duplicate fermentation

TABLE 5.—*Fermentation values with yeast extract (solution I), lead acetate [$Pb(Ac)_2$] precipitate (solution III), and lead acetate [$Pb(Ac)_2$] filtrate (solution II).*

Solution.	Ions added.	Titration value cc 0.1 *N* NaOH.	Comparative N content.	Titration value calculated to same N content.
cc extract in 150 cc total volume.				
50 cc solution I	None	(61.2) 59.5	0.120	(61.2) 59.5
Do	PO_4	70.0	0.120	70.0
Do	Mg	68.5	0.120	68.5
Do	Mg PO_4	(79.6) 82.5	0.120	(79.6) 82.5
50 cc solution II	None	32.5	0.078	49.5
Do	PO_4	55.5	0.078	85.0
Do	Mg	33.5	0.078	51.0
Do	Mg PO_4	50.0	0.078	76.5
50 cc solution III	None	(36.2) 36.5	0.046	(93.6) 94.5
Do	PO_4	53.0	0.046	138.0
Do	Mg	38.0	0.046	98.5
Do	Mg PO_4	58.0	0.046	150.0
50 cc solution I 50 cc solution II	None	103.0	0.198	62.5
Do	PO_4	115.5	0.198	70.0
Do	Mg	108.0	0.198	65.5
Do	Mg PO_4	124.0	0.198	75.0
50 cc solution I 50 cc solution III	do	129.0	0.166	93.0

values, in cases where they were taken, checked. Neither can these differences in fermentation value be due to possible precipitation of nitrogenous substances, since in some cases where heavy precipitates occurred after sterilization, fermentation values were very high; while in those cases where no precipitation occurred fermentation values were low. A more valid criticism would be that most of the gums having been included in the lead acetate precipitate might have been dissolved on treatment with sulphuric acid (H_2SO_4). The lead acetate precipitate fraction showed a reducing sugar content equivalent to 0.1 per cent, while the lead acetate filtrate gave 0.03 per cent glucose. Such small differences in glucose content over and above the original concentration of 2 per cent substrate cannot produce such considerable differences in fermentation value observed, especially in view of the fact that not all the original 2 per cent glucose is utilized even in those cases where maximum fermentation values are obtained. In Table 6, where fermentation value is determined from different original concentrations of sugar substrate, it can be seen that any small addition of sugar above 2 per cent has a negligible effect upon the fermentation value.

TABLE 6.—*Relation between titration value and original glucose concentration.*

[50 cc yeast extract adjusted to pH 7.1 before addition of glucose, H_2O, and final sterilization.]

Glucose added. Per cent.	Titration value. cc 0.1 *N* sodium hydroxide (Na OH).
0.0	1.5
2.0	60.0
5.0	69.0
8.0	73.5
15.0	76.5

Attention is called to the increasing effect of magnesium ion, of phosphate ion, and of a mixture of both on fermentation.

CONCENTRATION OF THE FERMENTATION FACTOR ACCORDING TO VITAMIN B_2 METHOD OF SEPARATION

Since the lead acetate separation shows that the potent substance is in its more concentrated form in the vitamin B_2 fraction, our next step was further to purify this fraction by the method of Euler(3) for vitamin B_2 separation.

The clear brownish solution III, obtained from the lead acetate precipitate, was treated with 15 per cent phosphotungstic acid dissolved in 5 per cent sulphuric acid (H_2SO_4) until precipitation was complete, and filtered. The amber-colored filtrate resulting from this is solution IV. In order to test the efficiency of removing the excess phosphotungstic acid, solution IV was divided into two portions. One portion was repeatedly shaken with iso-amyl alcohol until further extraction gave no test with zinc powder and concentrated hydrochloric acid (HCl) for the presence of phosphotungstic acid. The iso-amyl alcohol was then extracted with ether and the solution rid of ether by evaporation. The resulting solution, IV–b, was a bright clear yellow liquid. The other portion of solution IV was treated with a hot saturated solution of barium hydroxide [$Ba(OH)_2$] until definitely alkaline to litmus, and filtered. The resulting filtrate was a clear yellow solution (solution IV–a). Both solutions were then adjusted with sulphuric acid (H_2SO_4) to 0.1 *N* acidity, filtered, and portions of the filtrate used for experiments. As the higher potency of the fermentation factor was found in solution IV–b, as shown in Table 7, only this fraction was used in further purifications. Solution IV–b was then treated with 50 per cent silver nitrate ($AgNO_3$) to excess and filtered. The resulting filtrate was then carefully neutralized with sodium hydroxide (NaOH) and filtered. The resulting filtrate (solu-

tion VI) was colorless, but darkened on standing. The precipitate from this neutralization, on treatment with dilute hydrochloric acid (HCl) and filtering, yielded a filtrate (solution VII) that was clear, yellowish red, and highly fluorescent. Table 7 gives the fermentation value of the various fractions of purified yeast extract after adjusting the pH to 8 to 8.5. In all cases where yeast extract is used 50 cc were taken. This is true of all other fractions with the exception of solution VII, where 25 cc were used. The duration of fermentation was three

TABLE 7.—*Relation between titration value and N content of various vitamin B_2 purified fractions.*

Fraction used.	Titration value cc 0.1*N* NaOH.	Comparative N content.	Titration value corrected for salt concentration.	Corrected titration value calculated to same N content (0.139 per cent).
Solution I	(87.5)85.6	0.139	85.6	85.6
Solution I + Mg + PO_4	102.5	0.139	102.5	102.5
Solution II	46.5	0.073	48.4	92.0
Solution III	61.9	0.074	67.5	127.0
Phosphotungstic acid precipitate	19.0	0.086	19.2	31.0
Phosphotungstic acid precipitate + solution I.	116.0	0.225	117.4	72.5
Phosphotungstic acid precipitate + solution I + Mg + PO_4.	122.4	0.225	123.6	76.0
Solution IV–b	47.5	0.033	48.4	204.0
Solution IV–b + solution I	101.0	0.172	103.0	88.0
Solution IV–b + solution I + Mg + PO_4.	122.0	0.172	124.4	101.0
Solution IV–a	30.0	0.039	30.6	108.0
Solution IV–a + solution I	138.0	0.178	140.7	108.0
Solution IV–a + solution I + Mg + PO_4.	147.0	0.178	150.0	115.0
Acid $AgNO_3$ precipitate	9.0	0.011	9.2	116.0
Acid $AgNO_3$ precipitate + solution I	87.3	0.150	89.4	83.0
Solution VI	21.4	0.011	21.4	169.0
Solution VI + solution I	105.6	0.150	105.6	98.0
Solution VII	6.3	0.005	6.3	350.0
Solution VII + solution I	87.8	0.144	90.5	89.0
Solution VII + solution I + Mg + PO_4	112.0	0.144	115.3	114.0

weeks. Table 7 also verifies what has already been shown by Table 5, and further shows that those successive fractions proved to contain vitamin B_2 by rat tests are also the fractions which yield high fermentation values.

There is the possibility, however, that in the successive purifications of the yeast extract for vitamin B_2, certain amino acids favorable to the growth of the organisms are carried along with the vitamin, and due to their presence the titration values for

the purified fractions are increased. If this were true it would partly explain why the titration values (when calculated to the same nitrogen content) are almost the same as for those of yeast extract alone, without the purified extract.

On the other hand, the plotting of Van Niel's figures, showing the relation between titration values and nitrogen content for yeast autolysate and for peptone, and our figures for yeast extract (Table 10), show that while for peptone the titration value-nitrogen curve is linear for a wide range of nitrogen starting from zero, in the case of the yeast autolysate, the titration value increases suddenly with increasing nitrogen content and soon becomes a symptotic; our figures for yeast extract corresponding to a position between these two curves. These figures, then, would tend to show that the reason for no definitely increased titration values (calculated to the same N content), when our purified fraction was mixed with the original extract may be the fairly low concentration of the fermentation factor present in the purified extract as compared with the original yeast extract.

In order to settle this point, the amount of purified extract (solution VII) was varied, while the amount of original yeast extract was kept constant. Table 8 shows the effect of the concentration of solution VII on the titration value of the original

TABLE 8.—*Effect of varying concentrations of solution VII with some concentration of solution I on titration value.*

Solution I.	Solution VII.	Titration value (7 days) cc 0.1 *N* NaOH.	Titration value corrected for salt concentration.	Titration value difference per cc solution VII used.	Comparative N content.	$\frac{HP}{HAc}$
cc.	*cc.*					
25	0	34.5	34.5	----------	0.179	1:1.7
25	6	37.4	37.4	0.48	0.197	----------
25	10	56.8	57.9	2.34	0.210	----------
25	25	85.5	89.7	2.21	0.257	1:2.2
25	30	83.4	88.4	1.80	0.272	----------
0	25	22.2	23.3	----------	0.078	[a]

[a] Very little volatile acids.

yeast extract. As is shown in column 5 when only 6 cc of solution VII are used, the effect on the increase of titration value is 0.48 cc per 1 cc of solution VII. When 10 cc of solution VII are used, the titration value is increased to 2.34. When

25 cc are used, there is a slight decrease to 2.21, and when 30 cc are used there is a decrease to 1.80. If this change was simply due to the independent action of the organism on only the solution VII, the net increase per cubic centimeter of solution VII should be $\frac{23.3}{25}$ or about 1. When it is remembered that the concentration of the purified extract (solution VII) used in Table 7 is approximately only one-half of the concentration used in Table 8, which gave 0.48, it is clear why the effect of this extract in Table 7 was not so noticeable. Hence, it may be concluded that the effect of the purified extract is of enzymic nature and is shown very markedly only at certain high concentrations when mixed with the original yeast extract.

In this connection it may be pointed out that this purified extract (solution VII) seems to have the effect of inducing growth at a much earlier period. In the original yeast extract plus solution VII, growth subsides at the end of seven days, while in yeast extract alone the organisms remain alive even at the end of thirty days.

Another proof of the enzymic nature of solution VII may be obtained from the following experiment: An artificial medium was prepared containing in 300 cc the following:

	g.
D-glutamic acid	1.0
Arginine hydrochloride	0.5
Potassium hydrogen sulphate (K_2HPO_4)	1.0
Sodium chloride (NaCl)	1.8
Magnesium sulphate ($MgSO_4$)	1.09
Calcium chloride ($CaCl_2$)	1.03
Dextrose	6.0

The amino acids used were found to be present in fairly large quantities (in protein form) in cow's milk(6) and in yeast.(7) Fifty cc of this artificial medium were diluted to 150 cc in the usual way and used as a control. To another flask prepared in the same way 25 cc of solution VII were added and the solution diluted to 150 cc. After inoculation the flasks were incubated for thirty days. Table 9 shows the result of this experiment. It shows conclusively that while an artificial medium containing arginine and glutamic acid gave no growth or very little growth with propionic acid bacteria; when solution VII was added to the artificial media, propionic acid was formed and the ti-

TABLE 9.—*Incubation of artificial medium and solution VII.*

Media used.	Titration value (30 days).	Remarks.
Artificial (Al)	4.2	No odor of HP.
Al + solution VII	29.0	Distinct odor of HP, HAc.
Solution VII	6.3	Do.

TABLE 10.—*Relation between titrate V and per cent N of several media.*

Van Niel.				Our yeast extract.	
Peptone media.		Yeast autolysate.			
Per cent N.	Titration value.	Per cent N.	Titration value.	Per cent N.	Titration value.
0.9	11.2	0.5	28.7	0.14	9.5
0.32	8.0	0.16	27.2	0.028	1.7
0.08	3.3	0.09	24.4	0.006	0.3
0.016	0.7	0.02	16.8		

tration value was much greater than if solution VII alone were present as a medium.

CONCLUSION

While it has been shown that the potent substance responsible for increased fermentation of sugar by propionic acid bacteria is in the vitamin B_2 fraction, it cannot be proved at this stage of our work that vitamin B_2 itself is the potent substance. Ellinger and Koschara(8) have shown that water-soluble animal pigments (lyochromes), which they prepared from milk whey, contained vitamin B_2. Kuhn, György, and Wagner-Jauregg(9) have also demonstrated the presence of vitamin B_2 in their preparation of lactoflavins from milk whey, which they obtained by adsorption on fuller's earth in acid solution and elution of the adsorbate with a mixture of pyridin, methyl alcohol, and water; and Stern(10) has demonstrated that these flavins function as oxidation-reduction enzymes. Whether our fermentation factor is a flavin or vitamin B_2 or a mixture of these, and whether lactoflavin and vitamin B_2 are identical, remain to be proved.

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INDEX OF TEXTURE AND CLASSIFICATION OF PHILIPPINE SOILS

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INTRODUCTION

At present there are no accepted standard physical specifications for soils except the results of mechanical analysis. The physical properties of soils, as determined by mechanical analysis, have been used for years by the United States Bureau of Chemistry and Soils for the classification and mapping of soils of the United States.

As it is rather tedious to make a mechanical analysis, it would be very desirable to have some simpler method for acquiring the data necessary for soil classification.

Hardy has suggested a method whereby the moisture content of soils at the sticky point and also a fifth of the sand content can be represented by one value. This is called the index of texture and is expressed as follows:

$$\text{Index of texture} = \text{Moisture at sticky point} - \frac{\text{Per cent of sand}}{5},$$

$$\text{or} \qquad \text{I. T.} = \text{M. S. P} - \frac{\text{Per cent sand}}{5}.$$

A description of soils according to Hardy's texture index is given below.

Texture index.	Type of soil.
10 to 25	Sand.
25 to 40	Sandy loam.
40 to 50	Silt loam or loam.
50 to 55	Clay loam.
55 to 60	Heavy clay.

The texture index, which can be readily obtained, has been considered as a possible standard for the classification of soils. In order to test the accuracy of this suggested standard it would seem desirable to investigate a number of soils and obtain experimental data on the texture index and the mechanical analysis.

The investigation was undertaken to ascertain the accuracy of the texture index for classifying Philippine soils and also to determine what limitations it may have.

EXPERIMENTAL PROCEDURE

The materials used in this investigation consisted of thirty-eight samples of soils that were obtained from nine different excavations. Samples 1 to 10 came from Tagaytay Ridge, Cavite. The remaining samples were from Tuguegarao, Cagayan, a tobacco region. Both of these localities are in Luzon. A description of each soil sample is given in Table 1.

The mechanical analyses of these soils were made in accordance with the method of Olmstead, Alexander, and Middleton. For the determination of the texture index the procedure of Hardy was followed.

Data for the mechanical analyses are given in Table 2. In recording these results the sand separate was reported as total sand of the sample. As shown by the data the samples of soil selected for this work consisted of various textural types from coarse sandy loam to heavy clay and adobe clay. In the Tagaytay sandy loam type the surface soil is sandy loam, the subsoil is loam to clay loam, and the substratum is clay to adobe clay. Soils from Tuguegarao vary in textural grades from silty loam to silty clay.

In Table 3 are given the index of texture and the clay-sand ratio $\left\{\frac{\text{clay}}{\text{sand}}\right\}$ for the various soils examined.

The soil samples were separated into three groups in accordance with the index of texture. Group 1 (Table 4) consisted of soils that had a texture index below 36, the soils in group 2 (Table 5) had a texture index of 36 to 50, and those in group 3 (Table 6) a texture index above 50.

In comparing the data recorded in Tables 4, 5, and 6 with the descriptions of the soils given in Table 1 and the mechanical analyses in Table 2 we find the following:

Soil group.	Texture index.	Type of soil.
1	Below 36	Mostly sand, sandy, or silty loam.
2	36 to 50	Uncertain.
3	Above 50	Clay loam or heavy clay.

The soils in group 1 (Table 4) are all mostly sand, sandy, or silty loam. Those in group 3 (Table 6) are all clay loam or heavy clay.

Classification according to the texture index is uncertain for those soils included in group 2 (Table 5). For instance, sample 5 (Table 5) had an index of 44.8 and sample 16 an index of 44.9. The index was about the same for these two samples, but the mechanical analyses (Table 2) gave quite different results, as shown below:

	Sample 5. Per cent.	Sample 16. Per cent.
Sand	48.9	20.8
Silt	22.3	35.2
Clay	28.8	44.0

Sample 5 was a rather sandy soil, whereas sample 16 contained much less sand and considerably more clay than sample 5. This and similar examples illustrate the general trend of the results obtained for the soils in group 2 (Table 5).

Our data show that the texture index may, in general, be used for classifying Philippine soils that have a texture index below 36 or above 50. Between these limits the texture index is not a reliable criterion for classifying soils.

Classification of Philippine soils into different types in accordance with the texture index is quite different from the classification suggested by Hardy. Apparently the index of texture has only a limited application for Philippine soils.

SUMMARY

Thirty-eight samples of soils obtained from nine different localities were investigated.

For each of these soil samples a mechanical analysis was made, the index of texture determined, and the clay-sand ratio calculated.

The soil samples were separated into three groups according to the index of texture. Group 1 contained soils that had a texture index below 36, the soils in group 2 had an index of 36 to 50, and those in group 3 an index above 50.

From the results we have obtained it would appear that the soils in group 1 (texture index below 36) may be classified as mostly sand, sandy, or silty loam.

The soils in group 3 (index above 50) may be classified as clay loam or heavy clay.

Group 2 (index 36 to 50) contained soils that could not be classified exactly according to the texture index.

Apparently the index of texture has only a limited application for the classification of Philippine soils.

Classification of Philippine soils into different types in accordance with the texture index is quite different from the classification suggested by Hardy.

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TABLE 1.—*Descriptions of the soil samples.*

Sample No.	Depth of sample.	Morphological description.
	cm.	
1	0–24	Very dark brown to nearly black, granular, and friable sandy loam.
2	24–60	Very dark brown to nearly black, granular, and friable loam to clay loam.
3	60–100	Light reddish brown clay loam to clay; sticky when moist but friable when dry.
4	100–130	Yellowish brown adobe clay; adobe rock parent material of the subsoil.
5	0–15	Very dark brown to nearly black, loose, and granular sandy loam.
6	15–60	Light reddish brown clay loam; sticky when moist but friable when dry.
7	60–110	Yellowish brown adobe clay.
8	0–10	Dark brown to nearly black, coarse, granular, and loose and friable sandy loam.
9	10–60	Dark brown loam to clay loam; granular and slightly friable.
10	60–130	Light reddish brown clay loam to clay; heavy and sticky.
11	0–11	Grayish brown, loose and structureless silt loam.
12	11–15	Loose and granular silt loam; mottled brown, yellow, and gray.
13	15–20	Dark grayish brown, hard and brittle, heavy silt loam, mottled yellow and gray.
14	20–33	Dark grayish brown, brittle silty loam.
15	33–50	Hard and brittle silty clay loam; mottled yellow, brown, and purplish gray.
16	50–89	Mottled purplish brown and yellowish brown, brittle silt loam.
17	89–102	Mottled purplish brown and yellowish brown, friable silt loam.
18	102–120	Mottled purplish gray and yellowish brown, silty loam.
19	13–26	Dark brown, brittle silty clay with yellowish brown specks.
20	26–58	Dark brown, stiff clay or silty clay with yellowish brown streaks.
21	58–63	Dark brownish gray, friable and granular silty clay loam.
22	63–100	Mottled gray and yellowish brown, silty clay; partially decomposed.
23	0–30	Dark brown, friable silt loam.
24	30–43	Chocolate-brown with purplish tinge, friable silt loam, heavier than No. 23.
25	43–60	Light chocolate-brown with yellowish brown mottlings; silt loam to silty clay loam.
26	60–80	Light brown with yellowish brown mottlings, slightly friable, silty clay loam.
27	80–100	Light chocolate-brown with yellowish brown mottlings; silt loam to silty clay loam but light in texture.
28	10–30	Dark brown to light brown, massive and compact clay loam.
29	30–50	Light brown, granular, and slight friable clay with yellowish brown concretions.
30	50–100	Grayish brown, nearly compact silt loam.
31	0–24	Do.
32	24–49	Light yellowish brown, nearly compact silt loam.
33	49–70	Light yellowish brown, compact silt loam.
34	70–105	Yellowish brown, compact and fine, granular silt loam.
35	0–18	Gray, loose and granular, silty loam.
36	18–28	Grayish brown, loose and granular loam.
37	28–48	Yellowish brown, granular and nearly compact loam.
38	48–90	Yellowish brown granular loam, grading into fine sandy loam.

TABLE 2.—*The mechanical analyses of soil samples.*

Sample No.	Sand, 2 to 0.05 mm.	Silt, 0.05 to 0.005 mm.	Clay, 0.005 to 0.0 mm.	Sample No.	Sand, 2 to 0.05 mm.	Silt, 0.05 to 0.005 mm.	Clay, .005 to 0.0 mm.
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
1	49.8	24.3	26.0	20	26.2	21.9	51.9
2	25.0	28.5	46.5	21	26.5	22.3	51.2
3	25.5	21.8	52.7	22	41.1	15.6	43.3
4	35.6	16.7	47.7	23	13.8	50.2	36.8
5	48.9	22.3	28.8	24	11.7	48.6	39.7
6	28.3	27.2	44.5	25	11.1	35.0	53.9
7	31.7	29.9	38.4	26	14.0	43.3	42.7
8	41.9	26.6	31.5	27	26.9	42.7	30.4
9	11.9	35.5	52.6	28	31.0	29.3	39.6
10	15.0	33.5	51.5	29	21.2	37.1	41.7
11	17.8	46.4	35.8	30	17.6	31.7	50.7
12	17.6	42.2	40.2	31	46.4	30.8	22.8
13	14.1	44.7	41.2	32	43.1	33.0	23.9
14	18.6	36.6	42.8	33	53.3	26.8	19.9
15	14.7	41.1	44.3	34	43.2	34.7	22.1
16	20.8	35.2	44.0	35	49.0	32.4	18.6
17	34.5	24.9	30.6	36	42.3	28.3	29.4
18	40.7	32.9	26.4	37	43.7	22.5	33.8
19	27.6	21.6	50.8	38	48.0	21.1	31.2

TABLE 3.—*Clay-sand ratio* $\left\{\frac{clay}{sand}\right\}$ *and index of texture.*

Sample No.	Ratio, $\frac{clay}{sand}$.	Index of texture.	Sample No.	Ratio, $\frac{clay}{sand}$.	Index of texture.
1	0.52	37.0	20	1.98	50.6
2	1.86	61.0	21	1.93	50.0
3	2.06	71.7	22	1.05	42.2
4	1.33	68.8	23	2.66	43.2
5	0.58	44.8	24	3.39	47.1
6	1.57	63.1	25	4.85	49.2
7	1.21	62.2	26	3.05	46.6
8	0.74	44.1	27	1.13	41.4
9	4.42	75.7	28	1.27	36.4
10	3.43	74.8	29	1.96	40.0
11	2.01	41.6	30	2.88	62.6
12	2.28	41.8	31	0.49	31.7
13	2.92	45.7	32	0.55	33.2
14	2.30	45.2	33	0.37	30.3
15	3.01	46.0	34	0.51	32.7
16	2.12	44.9	35	0.37	30.4
17	0.88	35.2	36	0.69	32.8
18	0.64	35.7	37	0.77	32.3
19	1.84	49.8	38	0.65	30.4

TABLE 4.—*Soils having a texture index below 36.*[a]

[Group 1.]

Sample No.	Ratio, $\frac{\text{clay}}{\text{sand}}$.	Index of texture.	Sample No.	Ratio, $\frac{\text{clay}}{\text{sand}}$.	Index of texture.
17	0.88	35.2	34	0.51	32.7
18	0.64	35.7	35	0.37	30.4
31	0.49	31.7	36	0.69	32.8
32	0.55	33.2	37	0.77	32.3
33	0.37	30.3			

[a] These soils may be classified according to the texture index as mostly sand, sandy, or silty loam.

TABLE 5.—*Soils having a texture index of 36 to 50.*[a]

[Group 2.]

Sample No.	Ratio, $\frac{\text{clay}}{\text{sand}}$.	Index of texture.	Sample No.	Ratio, $\frac{\text{clay}}{\text{sand}}$.	Index of texture.
1	0.52	37.0	21	1.93	50.0
5	0.58	44.8	22	1.05	42.2
8	0.74	44.1	23	2.66	43.2
11	2.01	41.6	24	3.39	47.1
12	2.28	41.8	25	4.85	49.2
13	2.92	45.7	26	3.05	46.6
14	2.30	45.2	27	1.13	41.4
15	3.01	46.0	28	1.27	36.4
16	2.12	44.9	29	1.96	40.0
19	1.84	49.8			

[a] Classification of these soils according to the texture index is uncertain.

TABLE 6.—*Soils having a texture index above 50.*[a]

[Group 3.]

Sample No.	Ratio, $\frac{\text{clay}}{\text{sand}}$.	Index of texture.	Sample No.	Ratio, $\frac{\text{clay}}{\text{sand}}$.	Index of texture.
2	1.86	61.0	9	4.42	75.7
3	2.06	71.7	10	3.43	74.8
4	1.33	68.8	20	1.98	50.6
6	1.57	63.1	30	2.88	62.6
7	1.21	62.2			

[a] These soils may be classified according to the texture index as clay loam or heavy clay.

OBSERVATIONS ON THE LIFE CYCLE OF GNATHOSTOMA SPINIGERUM [1]

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THREE PLATES

INTRODUCTION

Human gnathostomiasis is unknown in the Philippines, although *Gnathostoma spinigerum* Owen has been observed frequently in the stomach of cats in this country (Tubangui, 1925). In Siam gnathostome infestation is not uncommon in man, as indicated by the five reported cases from that country (Levinsen, 1889; Leiper, 1909; Robert, 1922) and the seven new cases collected by Prommas and Daengsvang (1933) during a period of two years. This infestation has also been reported in man once from the Federated Malay States (Samy, 1918), thrice from China (Tamura, 1921; Morishita and Faust, 1925), once from Japan (Morishita, 1924) and twice from India (Maplestone, 1929; Datta and Maplestone, 1930). Curiously enough all the worms collected from human cases were immature and found in peripheral lesions.

The knowledge of the life cycle of *Gnathostoma spinigerum* is fragmentary. Chandler (1925) found larval gnathostomes encysted in the mesentery of the rock python (*Python reticulatus*), the king cobra (*Naja bungarus*), and the common cobra (*Naja tripudians*), which when fed to cats formed burrows in the liver or became embedded in the parietal peritoneal wall or renal capsules instead of developing into adult worms inside nodules in the stomach wall, but he was at a loss to establish the connection between these larvæ and the adult gnathostome. The first important contribution to the knowledge of the life cycle of this worm was furnished by Heydon (1929), who observed that the eggs of *Gnathostoma spinigerum* obtained from

[1] The work was made possible through a research grant from the Board of Regents, University of the Philippines. Received for publication January 15, 1936.

an infected cat, when kept in shallow water at room temperature developed inside fully motile larvæ in five days and, if kept in water up to twenty-three days, a certain proportion of them hatched out spontaneously embryos inclosed in large, loosely fitted, delicate sheaths. Evidently influenced by Heydon's work, Prommas and Daengsvang (1933) attempted to infect white rats by feeding them with fully developed embryonated eggs and newly hatched larvæ of *Gnathostoma spinigerum* with negative results. Skin penetration by newly hatched larvæ in experiments conducted on rats and mice did not occur. Attempts to infect *Ctenocephalus canis* and *C. felis* by allowing them to feed on *Gnathostoma* eggs containing fully developed motile embryos also gave negative results. These writers were, however, successful in infecting an apparently unidentified species of *Cyclops* with newly hatched motile larvæ of *Gnathostoma spinigerum.* The larvæ very soon pierced the gastric wall into the body cavity of the cyclopes after ingestion. In the body cavity of the crustacean, *G. spinigerum* larvæ underwent metamorphosis. Their size gradually increased and was 372.5 by 61.6 μ at 14 days of age. Fully developed larvæ, which showed structures similar to those found in the adult worm, were observed in the body cavity of the cyclopes in about seven days, although some may require a longer period. These results led the authors to conclude that "suitable and unsuitable hosts possibly get Gnathostome infestation by drinking water containing infected cyclops." Recently, however, we found gnathostome larvæ, presumably those of *G. spinigerum,* encysted in the muscles of three species of fresh-water fishes in Laguna de Bay, a lake about 25 kilometers south of Manila. As this finding promises to establish a natural and logical connection between the adult gnathostome in mammals and the experimental gnathostome larvæ in the *Cyclops* of Prommas and Daengsvang, we have decided to follow this clue. In this paper we are reporting the results of our preliminary investigation.

MATERIALS AND METHODS

Late in 1935, while searching for metacercariæ of heterophyid flukes in the muscle of some of our fresh-water fishes in connection with another work, we accidentally encountered in the flesh of *Glossogobius giurus* (Hamilton-Buchanan) (locally known as "bia") nine encysted gnathostome larvæ, which, judg-

ing from their size and structure, are presumably those of *G. spinigerum.* Subsequently the same larvæ were found in the muscle of *Ophicephalus striatus* Bloch ("dalag") and *Therapon argenteus* (Cuvier and Valenciennes) ("ayuñgin"). All of the eleven *Glossogobius giurus* examined were found to harbor the larvæ, an incidence of 100 per cent; of eight *Ophicephalus striatus* two were infected, an incidence of 25 per cent; and of twenty-three *Therapon argenteus* examined three yielded larvæ, an incidence of 12.6 per cent. All these fishes are abundant in Laguna de Bay. Cats and dogs imported from Biñan, a town on the shore of this lake, were autopsied to determine the presence of adult gnathostomes in these animals. In two of six cats autopsied, adult gnathostomes were found in typical nodules in the stomach wall without visible communication with the gastric cavity. Immature worms were also found buried in the livers of these two cats. The adult worms obtained from the stomach nodules answer faithfully the description of *Gnathostoma spinigerum,* even to the detail of the body spine as given by Faust (1929), except that our specimens possess nine transverse rows of cephalic hooklets instead of eight. However, according to Baylis and Lane (1920) the number of transverse rows of cephalic hooklets varies from eight to eleven in *Gnathostoma spinigerum.* Two of the seven dogs from the same town showed immature gnathostomes in the liver, identical with those found in the same organ of cats. A large number of cyclopes and small crayfishes were examined right in the town during a survey trip, but in no instance was a *Gnathostoma* larva encountered.

EXPERIMENTS

November 13, 1935.—Seven gnathostome larvæ obtained from the flesh of *Glossogobius giurus* were fed to a white rat which was killed nine days later (November 22). No worms were found in the stomach.

November 20, 1935.—Six gnathostome larvæ from *G. giurus* were fed to a white rat. The animal died November 28. The stomach was negative. Thinking that the larvæ might have gone to some peripheral focus, we included the liver in the examination, and two larvæ were found buried in this organ, causing a lesion similar to that described by Chandler in the livers of his experimental cats.

November 27, 1935.—Seven gnathostome larvæ from *G. giurus* were fed to a white rat, which was killed on December 7. One larva was found in what appeared to be a minute nodule in the stomach wall, and two were recovered from the liver.

December 16, 1935.—Nine gnathostome larvæ from *G. giurus* and seven from *Ophiocephalus striatus* were fed to a white rat, which was killed January 10, 1936. As the animal was killed twenty-four days after the infestation the possibility of finding larvæ in the muscles was considered. Six larvæ were found in the obliquus abdominis externus and posterior superficial pectoral muscles and one in the liver. There was no difference noted in either size or structure between the larvæ found in the muscle and the one in the liver.

December 31, 1935.—A survey trip to Biñan was made. A number of fishes of the species *G. giurus, O. striatus,* and *T. argenteus* caught in the nearby Laguna de Bay were examined and larvæ were found in each species. Two snakes examined were negative for gnathostome. Many cyclopes of different species were examined for gnathostome larvæ, but all were negative. Small shrimps and crayfishes were also examined, but were likewise negative.

Experimental infestation of cats has not been made because of the difficulty of finding animals that are absolutely free from infestation. Absence of eggs in the stool does not prove that a cat is free of this worm, because in some cases the nodule which contains the adult worms is devoid of an opening that would permit the free passage of eggs to the stomach lumen. We are expecting to conduct feeding experiments on cats in the near future, as we have several expectant cats which we hope will provide us with sufficient kittens free from infestation.

Experimental infestation of cyclopes has likewise not been made because we lacked infected cats that could supply us with eggs, but we hope soon to be able to perform this experiment to see if we can infect *Ophiocephalus striatus,* which can be cultivated in aquaria, with gnathostome larvæ by feeding it with infested cyclopes.

DISCUSSION

The success of Prommas and Daengsvang in infesting *Cyclops* with larvæ of *Gnathostoma spinigerum* and our discovery of *Gnathostoma* larvæ (presumably of *G. spinigerum* also) in the muscles of fresh-water fishes in a lake in the region around

which adult *G. spinigerum* appears to be a common natural infestation in the stomach of cats, promise an early solution of the life cycle of this worm. Although the possibility of direct infestation of suitable and unsuitable hosts by drinking water containing infected cyclopes as suggested by Prommas and Daengsvang cannot be entirely ruled out, infestation of these hosts by eating raw infested fish would appear to be the more natural and logical process, since the cat and the dog as well as human beings are by nature piscivorous. It would be interesting to know whether these authors have already succeeded in establishing infestations in these hosts by feeding them with infected cyclopes.

The size given by Prommas and Daengsvang for "mature" larvæ in their experimentally infected cyclopes is 0.3725 mm by 0.0616 mm. They remarked that no further change in structure was noted once this size was reached (which is on or about the seventh day) even when kept in the crustacean up to one month. Our gnathostome larvæ from fish muscles are considerably larger, having an average measurement of 4.5 mm by 0.312 mm, or approximately twelve times larger than the maximum development attained in the crustacean host (see Table 1). This would indicate that the larvæ undergo further development in the fish if we assume for the moment that the latter serves as the second intermediate host of *Gnathostoma spinigerum*. It would be easier and more logical to explain infestation of cats, dogs, and man by the ingestion of raw infected fish than by drinking water containing infected cyclopes, since, as has been pointed out, these hosts are by nature fish-eating; furthermore, it would be difficult to explain the light infestations usually observed in these mammalian hosts were they contracted by drinking water containing infected cyclopes. We observed that infestation in the fish is relatively light, the larval yield never exceeding nine in each fish in a total of forty-two dissections.

In India Chandler, as mentioned elsewhere in this paper, found gnathostome larvæ in the mesentery of snakes, which upon being fed to cats form burrows in the liver, or become embedded in the parietal peritoneal wall or the renal capsules instead of developing into adult worms inside nodules in the stomach. The larvæ in the cat, except for their considerably larger size, resemble the ones in the reptilian host in every other respect. Significantly the larvæ obtained by Chandler from the snakes approximate our larval gnathostome from the fish in size and

structure; furthermore, if these larvæ from the fish were fed to white rats much larger larvæ, which resemble in size and structure those recovered by Chandler from the liver of his experimental cats, could be found in the liver of white rats in from eight to ten days. By keeping the larvæ much longer (24 days) in white rats, larvæ could be obtained from the skeletal muscles of this animal. Morishita and Faust (1925) described larval gnathostome from the peripheral lesions in the human host which, like the ones recovered by Chandler from his experimental cats fed with larvæ of reptilian origin, differ from the adult *Gnathostoma spinigerum* in having only four instead of eight rows of transverse cephalic hooklets. Chandler suggests that the worms only attain full development after a final moult. Since the gnathostomes described by Leiper (1909) and Tamura (1921) from peripheral lesions in man were provided with a full compliment of hooklets, and in both size and structure were practically mature, one may be tempted to assume in a speculative manner that the worms of Chandler in the liver and peritoneum of his experimental cats and of Morishita and Faust in somatic foci in man could have developed, if they had been given sufficient time, to the semimature worms of Leiper and Tamura. We hope to verify this point by keeping the larvæ long enough in the body of our experimental animals. That gnathostomes need not necessarily be confined in the stomach of the cat is shown not only by the finding of Chandler but also by our own discovery of immature larvæ burrowing in the liver of naturally infested cats.

At this stage of our knowledge it is risky to speculate on the rôle played by snakes in the life cycle of the gnathostome. Do they get their infestation by eating infected fish, or do they infect themselves by drinking water containing infected cyclopes? Both are possible. However, the fact that the larvæ from the reptilian hosts approximate both in size and structure the larvæ from the flesh of fish would imply that snakes act in the same capacity as the latter animal. Having dissected so many snakes and obtained so many larvæ it is improbable that Chandler could have missed larger forms if these reptilian hosts allow further development of the worms in their bodies. Furthermore, snakes do not seem to include fish in their diet. On the other hand, it is still possible to find larger and structurally more developed larvæ in the snakes like those found by Morishita and Faust and by Leiper and Tamura in man. If this is found to be true,

the snakes would naturally assume the rôle of the so-called unsuitable hosts of *Gnathostoma* and not secondary intermediate hosts. An extensive dissection of reptilian hosts is necessary to clear this point. The possibility that snakes get infected with gnathostome larvæ by eating rats (and cats in the case of the python) is hardly tenable since the larvæ obtained by Chandler in these reptiles were smaller than those we found in the liver and muscles of experimentally infested rats and in the liver of a naturally infested cat. Besides, the larvæ in the snakes when fed to cats have been found by Chandler to undergo further development in the liver of the latter.

As regards the piscine hosts it appears more logical and natural that they get their infection by eating infected crustaceans, which are the natural food of fish. However, extensive search for infected cyclopes in Laguna de Bay, where infected fish abound, proved unsuccessful. Could this mean that cyclopes can be infected only when no other crustacean is present, as is the case under laboratory conditions; or, to put it in another way, is it possible that there are other crustaceans preferred by the hatched larvæ of *Gnathostoma* for their first development? Would they encyst in *Daphnea,* small shrimps, and other fresh-water crustaceans? We have made a preliminary survey of these possibilities with negative results. It would be interesting to put these possibilities to further tests.

SUMMARY

1. Cats obtained from Biñan, a town on the shore of Laguna de Bay, were found to harbor adult *Gnathostoma spinigerum* Owen contained in the stomach nodules. However, immature larvæ have been found to wander to somatic foci (liver) of a naturally infested cat.

2. Dogs from the same region are also naturally infested with this worm, but so far only immature worms in burrows in the liver have been found.

3. Encysted gnathostome larvæ of uniform size and structure (presumably of *G. spinigerum*) have been discovered in the muscles of three species of fresh-water fishes caught in Laguna de Bay; namely, *Glossogobius giurus* (Hamilton-Buchanan) (locally known as "bia"), *Ophiocephalus striatus* Bloch (locally known as "dalag"), and *Therapon argenteus* (Cuvier and Valenciennes) (locally known as "ayuñgin"). When these en-

cysted larvæ were fed to white rats, larvæ which seem to have undergone development both in size and structure developed in the liver and skeletal muscles of this animal.

4. Suitable and unsuitable hosts possibly get gnathostome infestation, not by drinking water containing infected cyclopes, but by eating raw fresh-water fish infested with larvæ which they possibly get by ingesting infected cyclopes.

5. Further studies of this subject are in progress.

ACKNOWLEDGMENT

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TABLE 1.—*Relative measurements of gnathostome larvæ.*

	From *Cyclops* of Prommas and Daengsvang. (Experimental.)	Natural infestation.				Artificial infestation.		
		From snakes of Chandler.	From flesh of our fish.	From liver of our puppy.	From liver of our cat.	From liver of Chandler's cat.	From liver of our white rat.	From muscle of our white rat.
Length mm	0.372	3–3.86	4.5	6.0	5.0	[b]	5.6	5.2
Width mm	0.0616	0.4–0.45	0.312	0.412	0.36	[c]	0.45	0.37
Globular cephalic swelling mm	[a]	0.21 by 0.1	0.2 by 0.12	0.24 by 0.13	0.23 by 0.11	[a]	0.22 by 0.13	0.19 by 0.75
Œsophagus:								
Length mm	[a]	[a]	1.437	1.62	1.55	[d]	1.69	1.4
Width at bulbous portion mm	[a]	[a]	1.187	1.287	0.25	[a]	0.27	0.18
Intestine length mm	[a]	[a]	2.5	2.58	2.75	[a]	3.5	2.91
Hooklets:								
Rows	4	4	4	4	4	4	4	4
Size μ	[a]	[a]	17 by 7	12.5 by 8	13.5 by 8	[a]	3 by 8	12.5 by 7

[a] Not given.
[b] 4 to 5.6 mm at two days; 6.2 to 6.85 mm at eleven days.
[c] 0.4 to 0.5 mm after two days; 0.52 to 0.57 mm after eleven days.
[d] 27 to 33 per cent of body length.

ILLUSTRATIONS

Plate 1

Fig. 1. Anterior end of *Gnathostoma* larva from the flesh of *Ophicephalus striatus* Bloch ("dalag"); low power.

2. The cephalic bulbar enlargement of the same larva, showing the detail of the four transverse rows of cephalic hooklets; high power.

Plate 2

Fig. 1. A section of the muscle of *Glossogobius giurus* (Hamilton-Buchanan) ("bia"), showing an encysted gnathostome larva; low power.

2. A section of the posterior superficial pectoral muscle of an experimental white rat, showing a gnathostome larva sagittally sectioned; low power. A part of the worm is not shown. Note that it has evidently grown both in size and structure.

Plate 3

Fig. 1. *Glossogobius giurus* (Hamilton-Buchanan) ("bia").

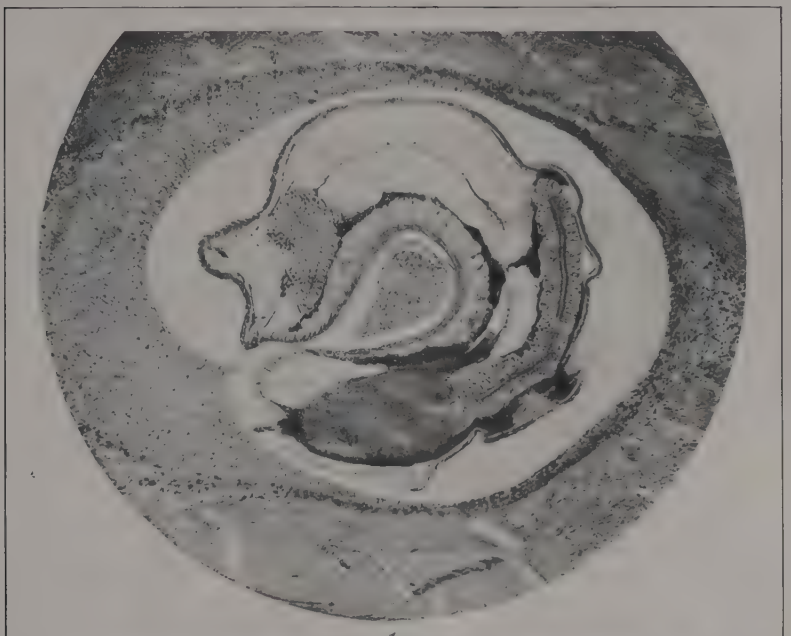
2. *Ophicephalus striatus* Bloch ("dalag").

3. *Therapon argenteus* (Cuvier and Valenciennes) ("ayuñgin").

1

2

PLATE 1.



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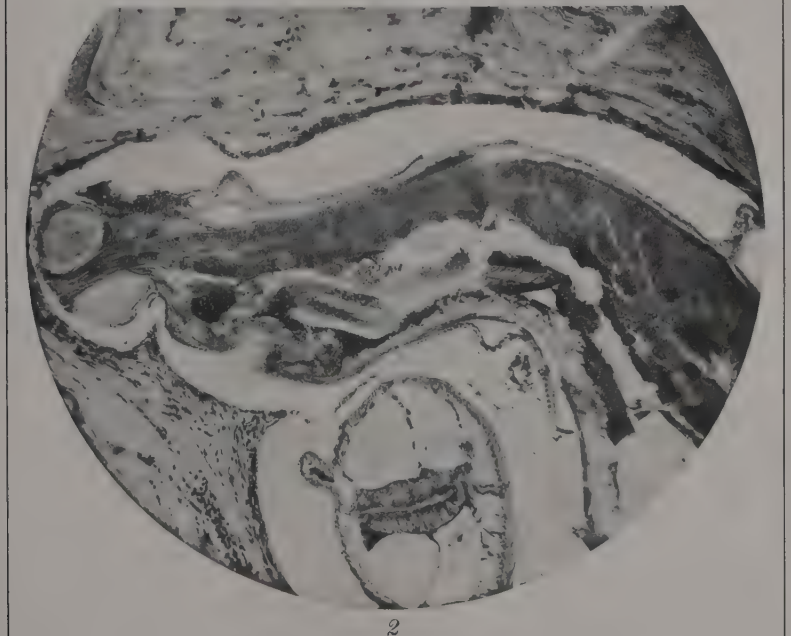
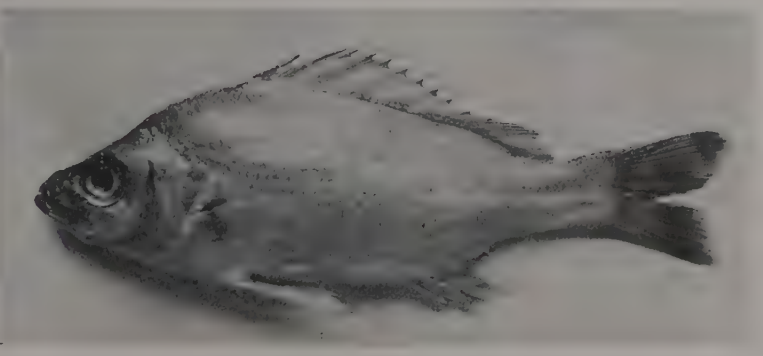


2

PLATE 2.

1

2



3

PLATE 3.

CHIRONOMIDÆ FROM JAPAN (DIPTERA), VI DIAMESINÆ [1]

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Of the Entomological Laboratory, Kyoto Imperial University, Japan

FOUR PLATES AND TWO TEXT FIGURES

The chironomid flies reported in the present paper were mainly collected at high altitude in the Japanese Alps (Plate 4, fig. 30) by Mr. K. Imanishi. In addition to these mountain species some specimens collected by myself at Kyoto are also described. The taxonomic system adopted in this report is based mainly on that of Edwards (1929), supplemented by that of Goetghebuer (1932).

I express here my hearty thanks to the director, Prof. Hachiro Yuasa, for his cordial help in this work. I am also greatly indebted to Mr. Kinji Imanishi for his continued interest in making known the rich chironomid fauna of the Japanese Alps. I am likewise indebted to Mr. Shigeo Hosono and Mr. Kiyoji Tsutsui for several mountain specimens.

The "antennal ratio" shows the relative length of the ultimate antennal segment to that of the remaining segments, excepting the scape, taken together. The "leg ratio" of the foreleg shows the relative length of the first tarsal segment to that of the tibia. Terminology for wing venation and corresponding abbreviations are given in Plate 1, fig. 5.

Key to the species of Japanese Diamesinæ.

[Genera and subgenera with asterisks have not been found in Japan.]

1. M_{3+4} anastomosing with Cu_1 at its deflecting part, m-cu absent. (*Prodiamesa* Kieffer.) ..... 2.
   M_{3+4} not anastomosing with Cu_1 at its deflecting part, m-cu present. 5.
2. Wings with macrotrichia at least on distal membrane. (Subgenus *Trichodiamesa* Goetghebuer.)*
   Wings without macrotrichia on membrane ..... 3.
3. Styles double ..... (Subgenus *Prodiamesa* Kieffer.)*
   Styles simple. (Subgenus *Monodiamesa* Kieffer.) ..... 4.

[1] Contribution from the entomological laboratory, Kyoto Imperial University, No. 50.

4. Coxites with double, setigerous, basal lobes; fourth tarsal segments cylindrical, longer than fifth; antennal ratio about 2.8.
P. (*M.*) *bathyphila* Kieffer.
Coxites with simple, nonsetigerous, basal lobes; fourth tarsal segments cordiform, shorter than fifth; antennal ratio about 0.1.
P. (*M.*) *brevitarsis* sp. nov.
5. Fourth tarsal segments cylindrical ..... 6.
Fourth tarsal segments cordiform or bilobate at tip..... 12.
6. Coxites produced far caudad beyond insertion of styles.
(*Protanypus* Kieffer.)*
Coxites not produced far caudad beyond insertion of style. (*Syndiamesa* Kieffer.) ..... 7.
7. Wings with macrotrichia at least on distal membrane. (Subgenus *Lasiodiamesa* Kieffer.) ..... *S.* (*L.*) *nivis* sp. nov.
Wings without macrotrichia on membrane. (Subgenus *Syndiamesa* Kieffer.) ..... 8.
8. Middle and hind tarsi with apical spurs on each of first two segments ..... 9.
Middle and hind tarsi with apical spurs on each of first three segments ..... 10.
9. Third segment of maxillary palpus distinctly produced beyond insertion of fourth segment ..... *S.* (*S.*) *takatensis* sp. nov.
Third segment of maxillary palpus not produced beyond insertion of fourth segment ..... *S.* (*S.*) *lanceolata* sp. nov.
10. Pronotum setigerous at least at side ..... 11.
Pronotum not setigerous ..... *S.* (*S.*) *kashimæ* sp. nov.
11. Fourth tarsal segment of foreleg longer than fifth.
S. (*S.*) *montana* sp. nov.
Fourth tarsal segment of foreleg shorter than fifth.
S. (*S.*) sp. (No. 1).
12. Dorsocentral hairs of thorax either absent or very small, decumbent, not arising from large punctures at their bases.
(*Heptagyia* Philippi.)*
Dorsocentral hairs of thorax present, long, suberect, arising from large punctures at their bases. (*Diamesa* Meigen.)..... 13.
13. Tibia of foreleg shorter than basitarsus; eyes bare.
(Subgenus *Potthastia* Kieffer.)*
Tibia of foreleg longer than basitarsus; eyes pubescent or bare..... 14.
14. Eyes bare. (Subgenus *Psilodiamesa* Kieffer.) *D.* (*P.*) *nigatana* sp. nov.
Eyes pubescent. (Subgenus *Diamesa* Meigen.) ..... 15.
15. Frontoclypeus bare; styles immovable ..... *D.* (*D.*) *astyla* sp. nov.
Frontoclypeus setigerous; styles movable ..... 16.
16. Eyes with minute dotlike pubescence ..... *D.* (*D.*) sp. (No. 2).
Eyes with normal long pubescence ..... 17.
17. Each of three proximal tarsal segments of each leg provided with apical spurs ..... 18.
Each of two proximal tarsal segments of each leg provided with apical spurs ..... 19.

18. Costa of wing produced beyond tip of R_{4+5}; antennal ratio of male 0.4 to 0.6 ..... *D.* (*D.*) *japonica* sp. nov.
    Costa of wing not produced beyond tip of R_{4+5}; antennal ratio of male 0.8 ..... *D.* (*D.*) *alpina* sp. nov.
19. Male antennæ not plumose; antennal ratio of female 0.4 to 0.5. *D.* (*D.*) *tsutsuii* sp. nov.
    Male antennæ plumose; antennal ratio of female more than 0.5.... 20.
20. Crossvein r-m distinctly curved ..... *D.* (*D.*) sp. (No. 1).
    Crossvein r-m almost straight ..... *D.* (*D.*) *plumicornis* sp. nov.

PRODIAMESA (MONODIAMESA) BATHYPHILA Kieffer.

KIEFFER, Ent. Mitteil. 7 (1918) 102; GOETGHEBUER, Faune de France 23 (1932) 151.

This fly was collected on a glass window in April at Shibutani, Higashiyama, Kyoto. The details of structure of this fly are little known, so a redescription of this species, based on the Japanese male specimen, is given below:

Body entirely black, 7 mm in length, distinctly setigerous in general appearance. Eyes extended narrowly dorsad around the antennal bases, not pubescent; antennæ 14-segmented, highly plumose with long dark setæ; antennal ratio 2.8; maxillary palpi 5-segmented (3 : 5 : 9 : 10 : 13). Pronotum distinctly separated into lateral halves, well developed, without setæ, each half pale brown on dorsomesal region; scutum slightly pruinose, with three shining vittæ, with paired setal rows on the pruinose areas between median and lateral vittæ, without median setal row; setæ all black, without pale basal punctures of the integument; supra-alar setal group represented by seven or eight small black setæ; humeral pits deep, elongate; scutellum black, highly setigerous; postscutellum also entirely black.

Abdomen beset with long black setæ which arise from pale basal punctures. Hypopygium (Plate 1, fig. 2) black, hairy; ultimate tergum setigerous on caudal margin with small setæ, provided with small anal point which is not strongly thickened, pubescent on proximal half; ultimate sternum very narrow; coxites large, elongate, hairy with many long setæ, each provided with about seven slender setæ on ventromesal chitinized ridge, a blunt, hairy projection on proximal region and two broad, fleshy, hairy projections on middle region; styles not double, with minute setæ, thickened on ventral side, each with a chitinized rodlet and two small spines at tip.

Legs brown, pale brown only at proximal end of each femur; fourth tarsal segments cylindrical, longer than fifth; fore tibia with a spur which is slender, bare, slightly longer than diameter of tibial end; tarsal segments of foreleg without apical spurs; proportional lengths of segments of foreleg, excepting coxa and trochanter, showing 80 : 105 : 87 : 83 : 31 : 20 : 12; leg ratio about 0.8; middle and hind tibiæ each with two spurs of which the longer one is as long as diameter of tibial end; comb of hind tibia consisting of about eleven spines; proximal two tarsal segments of middle and hind legs each with two, strong, apical spurs; proportional lengths of distal two tarsal segments of hind leg 17 : 7; claws strong, slightly spatulate and obscurely pectinate at end.

Wings hyaline, without macrotrichia, slightly milky white by reflected light; anterior veins and stem of M and Cu_1 much darkened; costa produced beyond end of R_{4+5}; R_{2+3} ending on costa at middle between ends of R_1 and R_{4+5}; m-cu absent; M_{3+4} and Cu_1 anastomosing for a short distance. Halteres yellowish white; stems brown.

Specimen.—Alcoholic male; wing, legs, and hypopygium mounted in euparal; Shibutani, Higashiyama, Kyoto; April 4, 1932; deposited in the entomological laboratory of Kyoto Imperial University; collected by M. Tokunaga.

This Japanese specimen is somewhat different from the European specimens in the coloration of various parts, but these differences may be local variations.

PRODIAMESA (MONODIAMESA) BREVITARSIS sp. nov.

This fly was captured at a light screen set by a rapid stream at Kibune, Kyoto.

Male.—Body entirely black, about 2.8 mm in length; wings about 2 mm in length; halteres yellowish brown. Eyes bare; antennæ (text fig. 1, *b*) 14-segmented; plumose hairs highly reduced; distal antennal segment very short, shorter than preceding two segments taken together, distinctly swollen distally, pubescent with small hairs; second to fourth antennal segments very short, somewhat discoidal; sixth to thirteenth segments elongate, fusiform; antennal ratio about 0.1 (4:30). Setæ of thoracic tergum small, without basal punctures on integument; pronotum with several setæ on each lateral side. Hypopygium (text fig. 1, *a*) black, without anal point; coxites slender, elongate, each with a chitinized basal lobe; styles small, not elongate, pubescent, with small setæ, each with a minute, apical

spine; ultimate tergum without strong setæ, but pubescent with minute hairs on caudal part, provided with a pair of membranous lobes on caudal margin; ultimate sternum without setæ, with a small membranous lobe on caudal margin between bases of the coxites.

Legs dark brown; tibiæ paler than other segments; tarsal spurs wanting in all legs; foreleg with one tibial spur and other legs with two; fourth tarsal segment of each leg broad, bilobate at tip, very small, half as long as fifth; relative lengths of segments of legs, excepting two proximal segments, as follows: 37 : 50 : 21 : 11 : 6 : 3 : 6 in foreleg, 47 : 48 : 23 : 13 : 7 : 3 : 6

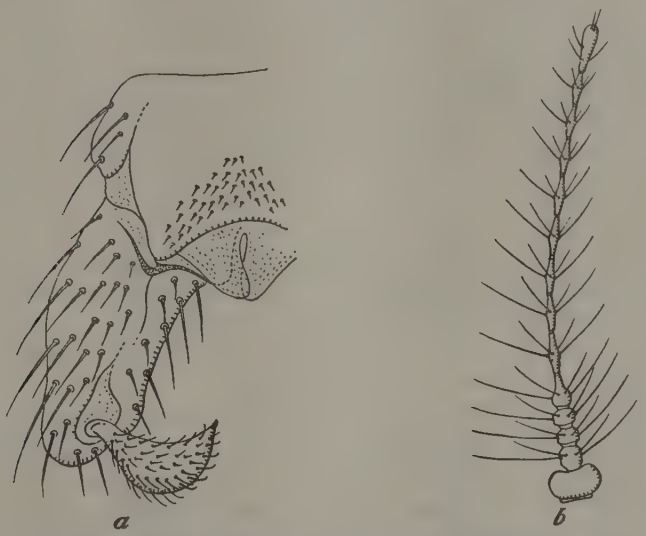


FIG. 1. *Prodiamesa brevitarsis* sp. nov.; *a*, male hypopygium, dorsal aspect; *b*, male antenna.

in middle leg, and 50 : 59 : 32 : 18 : 8 : 3 : 7 in hind leg. Wings comparatively broad, brown by transmitted light, without macrotrichia on membrane; costa produced beyond end of R_{4+5}; anastomosis between M_{3+4} and Cu_1 distinct.

Habitat.—Kyoto, Japan.

Holotype.—Alcoholic male; Kibune, Kyoto; October 16, 1934; deposited in the entomological laboratory of Kyoto Imperial University; collected by M. Tokunaga.

The present species does not show certain generic characters of *Prodiamesa* given by Edwards and Goetghebuer, especially the short fourth tarsal segments, and rather resembles *Hep-*

tagyia. I decided, however, to place it under the subgenus *Monodiamesa* of the genus *Prodiamesa*, owing to the important character of the wing venation. This fly is easily distinguishable from the other related species by the extremely small value of the antennal ratio and the short, bilobate, fourth tarsal segments.

SYNDIAMESA (SYNDIAMESA) LANCEOLATA sp. nov.

This fly was captured in the botanical garden of Kyoto Imperial University, Kitashirakawa, Kyoto, in early spring.

Male.—Body about 4.5 mm in length, entirely black, hairy in general appearance. Eyes bare, extending dorsad around antennal bases and very narrowly separated from each other on dorsal side; frontoclypeus black, hairy; antennæ 14-segmented, highly plumose with dark hairs, each with a short apical seta; antennal ratio about 2 (45 : 22); scape with three, short, black setæ; maxillary palpi distinctly 5-segmented (3 : 5 : 11 : 13 : 16). Pronotum well developed, separated at middle by a deep V-shaped incision, setigerous on ventrolateral and dorsomesal margins; highly pruinose, with black setæ along pseudosutural foveæ, without middorsal setæ; supra-alar setal group consisting of about fourteen black setæ; scutellum black, setigerous all over the surface; postscutellum entirely black, bluntly projecting dorsad at caudal end; thoracic setæ not arising from pale basal punctures.

Abdomen entirely black, with numerous pale setigerous punctures. Hypopygium (Plate 1, fig. 1) black, setigerous; ultimate tergum broad, subdivided by a V-shaped chitinization into a median, triangular, nonsetigerous area and paired, lateral, setigerous areas; anal point long, sharply pointed, needlelike, slightly lanceolate, hyaline on distal half; ultimate sternum subdivided into paired, setigerous hemisternites; coxites also setigerous, broad, each with a fleshy, setigerous projection on proximal side; styles extending straight, beset with short setæ, each with a minute black spine within a minute apical concavity.

Legs entirely brownish black; fourth tarsal segments of all legs cylindrical; foreleg with the following proportional lengths of segments: 85 : 105 : 82 : 41 : 26 : 12 : 10; leg ratio about 0.8; tibial spur of foreleg pubescent, very slightly longer than diameter of tibial end; fore tarsal segments without apical spurs; tibiæ of middle and hind legs each with two, apical, pubescent spurs, those of middle leg subequal in length to each other, those of hind leg unequal, the mesal being longer than the other and

slightly longer than diameter of tibial end; tibial comb of hind leg consisting of about twelve strong setæ; proximal two tarsal segments of middle and hind legs each provided with two apical spurs; distal two tarsal segments of hind leg subequal in length. Wings without macrotrichia; costa distinctly produced beyond end of R_{4+5}; m-cu present, very short; squama brownish black, with brown fringe. Halteres entirely dark brown.

Habitat.—Kyoto, Japan.

Holotype.—Alcoholic male; legs and hypopygium mounted in euparal; March 31, 1932; deposited in the entomological laboratory of Kyoto Imperial University; collected by M. Tokunaga.

Although this species is slightly different from the other known members of the genus *Syndiamesa* in the setigerous pronotum and absence of the apical spurs of third tarsal segments of all legs, in other structures it is similar to *S. vidua* Kieffer and *S. nivicola* Bezzi, excepting the characteristic value of the antennal ratio and the proportional lengths of the leg segments.

SYNDIAMESA (SYNDIAMESA) TAKATENSIS sp. nov.

This description is based on a single specimen of a male fly found in the collection made on snow in spring at Takata, Nigata Prefecture, and labeled *Chironomus takadensis* Matsumura, collectively, for four different insects; namely, two species of *Spaniotoma, Diamesa japonica,* and the present fly. The species incorrectly named by Matsumura is thought to be a species of *Spaniotoma,* judging from the wing venation figured by Sueo Izumi.[2]

Male.—Body about 5.8 mm in length, entirely black, not so highly hairy as in the former species. Eyes bare, reniform, widely separated from each other on dorsal side; distance between them subequal to vertical lengths of eyes, or 0.42 times as wide as head width. Antennæ 14-segmented, highly plumose; antennal ratio about 2.84. Maxillary palpi 5-segmented (4 : 6 : 15 : 17 : 18); middle segment distinctly produced dorsodistad beyond insertion of following fourth segment. Pronotum setigerous only at side; scutum setigerous; supra-alar setal group represented by about sixteen black setæ; thoracic setæ arising from pale punctures. Hypopygium black, setigerous; ultimate tergum with a small, needlelike, black, anal point; coxites slender, without basal lobes, not distinctly produced beyond insertion

[2] Insects on snow. Investigations of snow, No. 1 (1929).

of styles; styles comparatively slender, each with a long black spine and many strong apical bristles.

Legs entirely black; fore tarsal segments broken off; proximal two tarsal segments of middle and hind legs each provided with two apical spurs; fourth tarsal segments cylindrical, longer than ultimate segments, and showing the following proportional lengths between them: 10 : 9 in middle leg and 12 : 10 in hind leg. Wings and halteres as in the former species.

Habitat.—Takata, Japan.

Holotype.—Alcoholic male; deposited in the entomological laboratory of Kyoto Imperial University; data of collection obscure on label.

This species is related to the former species, but distinctly different in the characteristic structure of the maxillary palpi, widely separated eyes, and structure of the hypopygium.

SYNDIAMESA (SYNDIAMESA) MONTANA sp. nov.

This species was captured at Tsurugisawa in the Japanese Alps, altitude about 2,400 to 2,700 meters.

Male.—Body length about 5.3 mm; wing expanse about 9 mm; color entirely dark brown. Head small, 0.75 mm in width, beset with short black setæ; eyes reniform, bare, widely separated from each other; antennæ highly plumose, 14-segmented; distal segment elongate, slightly clavate, with an apical hair; antennal ratio about 1.65; scape large, with a sensory pore and three small setæ; maxillary palpi 5-segmented, including small, proximal, nonsetigerous segment (4 : 7.5 : 13 : 14.5 : 16); third palpal segment somewhat swollen ventrad, with a sensory pore.

Thorax brownish black; pronotum setigerous only at side; scutum without middorsal setæ; setæ along pseudosutural foveæ directly arising from dark integument; scutellum setigerous all over the surface. Abdomen dark brown, with brown hairs on dorsal side. Hypopygium (Plate 1, fig. 4) setigerous; ultimate tergum with a short cylindrical anal point, without distinct hairs; coxites large, each with a small setigerous projection on distomesal margin; styles large, each with a tiny tooth at tip.

Legs entirely brown; claws slightly spatulate, each with seven minute teeth at tip; two tibial spurs on each middle and hind leg and one on foreleg; these spurs sharply pointed, large, swollen on proximal region, pubescent basally; tibial comb of hind leg composed of about sixteen strong setæ; proximal three tarsal segments of each leg provided with apical spurs; segments of hind leg showing the following proportional lengths:

123 : 141 : 94 : 47 : 36 : 16 : 14; leg ratio about 0.66; fourth tarsal segment of middle and hind legs shorter than fifth, the ratio being 11 : 13 in middle leg and 14 : 15 in hind leg. Wings hyaline, slightly brown, without macrotrichia; anal lobe almost rectangular, squama thickly fringed; main veins brown; Sc ending on costa at opposite side of end of Cu_1; R_{2+3} extending along center between R_1 and R_{4+5} ending nearer R_{4+5} than R_1; relative lengths of R_1 and R_{4+5} about 16 : 27; r-m thrice as long as first section of M_{3+4}, strong, oblique, straight; m-cu one-third as long as first section of M_{3+4}; costa produced along wing margin beyond end of R_{4+5}; this prolongation one-fourth as long as distance between ends of R_{4+5} and M_{3+4}. Halteres yellowish white.

Male pupa.—Body about 6.5 to 7 mm in length; exuviæ brown; abdomen entirely covered with minute dotlike spinules, without tubercles and swimming hairs. Head with a blunt median projection on vertex, one pair of strong setæ on vertex behind antennal bases and two pairs of small setæ on marginal ridges of genæ behind the regions of compound eyes. Thorax with a long seta on each lateral half of pronotal region, one pair of double setæ on cephalic margin of scutal region, three long setæ on lateral side caudad of pronotum, two short setæ on middle of each pseudosutural fovea; pronotal respiratory organs completely atrophied, being represented by a pair of depressions of integument; this peculiar feature of the respiratory organs is also known in *Diamesa lurida* Garrett. Each abdominal segment with thickly chitinized bands on cephalic margin, well-developed lateral expansions; these lateral expansions without pointed chitinizations on margins but each with a dark rudiment of a spiracle and four setæ; these setæ of lateral expansion all slender, very small on first five abdominal segments; one seta on ventrocaudal margin of lateral expansion of sixth segment finely branched; all setæ of seventh and eighth expansions distinctly branched (Plate 3, fig. 29); venter of each abdominal segment provided with two pairs of small setæ and dorsum, excepting that of eighth, with four pairs of minute and one pair of slender setæ; ultimate segment (Plate 3, fig. 28) semicircular from dorsal aspect, deeply incised at caudomesal margin, with paired dark tubercles and setal groups, each of which consists of three very long slender setæ on caudal margin; one pair of branched setæ on margin of a V-shaped caudal incision; genital sheaths deeply bilobate, extending beyond caudal margin of ninth segment between tubercles.

In general appearance the present pupa somewhat resembles that of *Syndiamesa branickii* Nowicki, but is distinctly different in the absence of filiform prothoracic respiratory organs, absence of dorsal chitinized patches of anterior abdominal segments, and presence of the comparatively long anal setæ.

Habitat.—Japanese Alps, Japan.

Holotype.—Alcoholic male; legs mounted in euparal; Tsurugisawa, Toyama Prefecture; October 17, 1928; deposited in the entomological laboratory of Kyoto Imperial University; collected by Mr. K. Imanishi.

The setigerous pronotum and shorter fourth tarsal segments of the middle and hind legs are thought to be peculiar for this genus but not rare among the Japanese species of *Syndiamesa*. This species is related to *Syndiamesa hygropetrica* Kieffer and *S. macronyx* Kieffer in the structure of the legs and hypopygium, respectively, but in the structure of the antennæ distinctly different from these related species.

SYNDIAMESA (SYNDIAMESA) KASHIMÆ sp. nov.

This fly was found on the spring snow at Kashima, Japanese Alps, Nagano Prefecture, about 1,600 meters in altitude.

Male.—Body length 5.7 mm; wing expanse 9 mm; thorax dark brown, abdomen and legs pale brown (immature?). Head large about 1 mm in width, entirely dark brown, including antennæ and mouth parts; occiput setigerous with black setæ; eyes bare, widely separated from each other; frontoclypeus with many long dark hairs; antennæ 14-segmented, densely plumose with long black hairs; scape with two setæ on ventral side; proximal eleven segments of flagellum very short, ring-shaped, each with about fifteen long plumose hairs; distal segment with a short apical seta; antennal ratio 3 (60 : 21); maxillary palpi 5-segmented (4 : 11 : 17.5 : 21 : 22).

Pronotum quite bare; scutum with many long black setæ along pseudosutural foveæ, without middorsal setæ; scutellum pale brown, highly setigerous. Abdomen highly setigerous with long black setæ on dorsum, nonsetigerous on venter. Hypopygium (Plate 1, fig. 3) distinctly darker, more setigerous than other segments; distinct projection of coxites wanting; styles comparatively slender, each with three tiny teeth on distal margin, without distinct bristles; ultimate tergum membranous, setigerous, with broad, pubescent, anal point.

Legs pale brown, highly setigerous; coxæ, trochanters, distal ends of all leg segments, articulations between femora and tibiæ,

and distal tarsal segments of all legs black; proximal three tarsal segments of all legs with apical spurs; tibial spurs and combs similar to those of the other species; claws each with one small seta and four or five large setæ at base, serrate into six minute teeth at tip; leg ratio of foreleg about 0.66; relative lengths of segments of foreleg 104 : 131 : 86 : 47 : 35 : 17 : 15; ultimate tarsal segments of middle and hind legs longer than penultimate, their ratio being 12 : 11 in middle leg and 15 : 14 in hind leg. Wings hyaline, without macrotrichia; anal lobe well developed; squama fringed with long setæ; main veins brown; Sc ending on costa a little before the opposite side of end of Cu_1; costa produced beyond end of R_{4+5}; this prolongation one-third as long as distance between ends of R_{4+5} and M_{1+2}; R_{2+3} extending closer to R_{4+5} than to R_1; r-m straight, about thrice as long as first section of M_{3+4}; m-cu very short, half as long as first section of M_{3+4}. Halteres white.

Habitat.—Japanese Alps, Japan.

Holotype.—Alcoholic male; legs mounted in euparal; Kashima, Nagano Prefecture; April 1, 1928; deposited in the entomological laboratory of Kyoto Imperial University; collected by Mr. K. Imanishi.

This species resembles *Syndiamesa hygropetrica* Kieffer in the structure of the male hypopygium, but differs distinctly in the narrow, ring-shaped, intermediate, flagellar segments of the antennæ.

SYNDIAMESA (LASIODIAMESA) NIVIS sp. nov.

This species is often found on the spring snow and has been captured at various places from an altitude of 900 to 1,300 meters in the Japanese Alps.

Female.—Body length about 6.8 mm; wing expanse about 14 mm; general color black. Head capsule reniform in frontal aspect, 0.84 mm in width; eyes bare, occupying two-thirds of head, widely separated from each other; frontoclypeus highly setigerous with long silky white hairs; antennæ 7-segmented (6 : 7 : 5.2 : 5 : 4.5 : 4.3 : 13.5), with long stiff verticils; scape with five setæ on ventral side; pedicel pale on proximal part, with many verticils arranged in two rings; intermediate segments of flagellum, from first to fourth, elongate, barrel-shaped, each with four to six stiff verticils; distal segment elongate, fusiform, with one short verticil on dorsal side and three apical hairs, equal in length to preceding three segments taken together;

maxillary palpi large, far longer than antennæ, 5-segmented (4 : 10 : 16 : 19 : 24). Pronotum setigerous on both dorsal and lateral sides; scutum with many setæ along pseudosutural foveæ, without middorsal setæ; scutellum beset with long silky hairs. Abdomen dark brown, setigerous; ultimate sternum also highly setigerous; ultimate tergum subdivided into paired setigerous hemitergites by a median membrane; cerci large, elongate, and curved ventrocephalad; spermathecæ three, discoidal, without colored ducts or necks.

Legs entirely dark brown; tibial spurs as usual in other members of this genus; tibial comb of hind leg consisting of about seventeen large bristles; foreleg with the following proportional lengths of segments: 120 : 150 : 102 : 57 : 42 : 22 : 20; leg ratio about 0.68; all tarsi provided with apical spurs on each of proximal three tarsal segments; fifth tarsal segment of middle leg equal in length to fourth (16 : 16) and that of hind leg slightly shorter than fourth (19 : 20). Wings hyaline, with macrotrichia on distal parts of cells R_5, M_2, and M_4; squama densely fringed; anal lobe almost rectangular; main veins brown; Sc ending on costa at opposite side of end of Cu_1; R_{2+3} ending at middle of wing margin between ends of R_1 and R_{4+5}; relative lengths of R_1 and R_{4+5} about 3 : 5; m-cu half as long as first section of M_{3+4}; r-m straight, thrice as long as first section of M_{3+4}; costa very slightly beyond end of R_{4+5}. Halteres yellow.

Habitat.—Japanese Alps, Japan.

Holotype.—Female; wing and legs mounted in euparal; Haruizawa, Nigata Prefecture; April 2, 1928.

Paratypes.—Females; Tsubame and Sasagamine, Nigata Prefecture, and Kashima, Nagano Prefecture; March 29 and 30 and April 1, 1928.

Type specimens.—Alcoholic; deposited in the entomological laboratory of Kyoto Imperial University; collected by Mr. K. Imanishi.

This species resembles *Syndiamesa borealis* Kieffer, but differs in the presence of the macrotrichia on the wing membrane and in the elongate, barrel-shaped, antennal segments. The macrotrichia are also present on the wings of *S. pilosa* Kieffer, but in this species they are found only at the tip of cells R_5 and M_2.

SYNDIAMESA (SYNDIAMESA) sp. (No. 1).

This fly was captured at Sasagamine, Japanese Alps, about 1,300 meters in altitude.

Female.—Body about 5.5 mm in length, ground color black; wing expanse about 12 mm. Eyes bare, widely separated from

each other; distance between them more than one-third of head width on dorsal side; antennæ 8-segmented, with many black verticils; scape with four setæ on ventral side; pedicel elongate, barrel-shaped, with about twelve hairs; proximal five segments of flagellum subequal in length to each other; each segment of flagellum, excepting distal one, somewhat pitcherlike; distal segment as long as preceding two segments taken together, fusiform, with two apical setæ; antennal ratio about 0.35. Pronotum setigerous at side. Wings without macrotrichia; costa slightly produced beyond end of R_{4+5}; relative lengths of veins R_1 and R_{4+5} about 30 : 54; r-m straight, four times as long as first section of M_{3+4}. Tibial comb of hind leg composed of about nineteen setæ; fourth tarsal segments of all legs shorter than fifth, being 15 : 19 in foreleg, 11.5 : 17.5 in middle leg, and 15 : 19 in hind leg; leg ratio of foreleg about 0.63; proximal three tarsal segments of each leg with apical spurs. Hypopygium darker than other abdominal segments; cerci triangular, pointed caudad; eighth sternum setigerous, with a deep caudal incision; ninth tergum thickened at side, with soft setæ; spermathecæ three, agreeing with those of *Syndiamesa nivis*.

Habitat.—Japanese Alps, Japan.

Specimen.—Alcoholic female; legs mounted in euparal; Sasagamine, Nigata Prefecture; March 26, 1928; collected by Mr. K. Imanishi.

This fly is allied to *Syndiamesa macronyx* Kieffer, but is distinctly different in the black coloration.

DIAMESA (PSILODIAMESA) NIGATANA sp. nov.

This fly was captured on the spring snow at Sasagamine, Nigata Prefecture, Japanese Alps, about 1,300 meters in altitude.

Male.—Body about 6 mm in length, ground color black. Head dark brown, including antennæ, mouth parts, and maxillary palpi; eyes bare, reniform, widely separated from each other; antennæ highly plumose, 14-segmented; antennal ratio about 3.35; maxillary palpi 5-segmented, including small, basal, nonsetigerous segment (3 : 6 : 10 : 12 : 20).

Thorax deep black; pronotum slightly setigerous at side; scutum slightly pruinose, with paired, lateral, setigerous lines along pseudosutural foveæ, without middorsal setæ; setæ of scutum not arising from pale basal punctures of integument; supra-alar setal group represented by about ten black setæ. Both sides of abdomen dark brown, thickly beset with long black setæ, which

arise from pale basal punctures. Hypopygium (Plate 2, fig. 10) simple, setigerous; ultimate tergum membranous, setigerous only on lateral halves, without anal point; coxites elongate, setigerous, projected mesad on ventroproximal edge, without other distinct setigerous projections; styles also beset with small setæ, flattened, each with a minute, apical tooth and several minute, branched setæ on apex.

Legs entirely dark brown; proportional lengths of segments of foreleg 76 : 87 : 60 : 36 : 21 : 11 : 9; leg ratio about 0.69; tibia of foreleg with an apical spur, which is basally pubescent and as long as diameter of tibial end; first tarsal segment of foreleg with apical spurs; second tarsal segment with preapical spurs a little before tip; other three tarsal segments of foreleg without spurs; tibiæ of middle and hind legs each with two basally pubescent spurs, of which a longer one in the case of the middle leg is subequal to, and in the case of the hind leg is slightly longer than, diameter of tibial end; comb of hind leg consisting of about fourteen strong bristles; proximal three tarsal segments of middle and hind legs each with small spurs on ventral side in addition to apical spurs; fourth tarsal segment of each leg somewhat longer than fifth, slightly constricted beyond middle, distinctly flattened to an oval shape distad of the constriction; ratio between two distal segments of hind tarsus about 14 : 12, fourth being longer than fifth. Wings hyaline, without macrotrichia on membrane; m-cu very short; r-m curved; R_1 and R_{4+5} not swollen. Halteres dark brown.

Habitat.—Japanese Alps, Japan.

Holotype.—Male; head, legs, wings, and hypopygium mounted in euparal; Sasagamine, Nigata Prefecture; March 9, 1932.

Type specimen.—Alcoholic; deposited in the entomological laboratory of Kyoto Imperial University; collected by Mr. K. Imanishi.

This fly is of an intermediate form between the genera *Syndiamesa* and *Diamesa*, having the following characters of the former genus: Fourth tarsal segment longer than fifth and three proximal segments of the middle and hind tarsi spurred. This fly has the following characters of the latter genus: Setigerous pronotum, flattened fourth tarsal segments, and two proximal segments of the fore tarsus spurred; but this fly is placed in *Diamesa*, and not in *Syndiamesa*, owing to the important character of the flattened fourth tarsal segments. These intermediate characters will serve to distinguish the present species from other related species of *Diamesa*.

DIAMESA (DIAMESA) ALPINA sp. nov.

This species was captured at Tsurugisawa, Japanese Alps, 2,400 to 2,700 meters in altitude (Plate 4, fig. 30), on the new snow in October and at Kakogawa, 1,500 to 1,600 meters in altitude, on the spring snow. The adults of this species are commonly found on the mountain snow in association with a certain nival stonefly, *Capnia nivalis* Ueno. Sometimes many larvæ, pupæ, and imagines of this species were found in the stomach of a torrential fish, *Salvelinus pluvius*, captured in Kashima River, 1,000 to 1,500 meters in altitude, October 13, 1930. The gelatinous cords of egg masses were found along the stream adhering to various substrata, such as wet stones, twigs, etc. The eggs are arranged in two longitudinal lines within the gelatinous matrix (Plate 4, fig. 32). Among the material taken from the stomach of a fish, I found many copulating pairs of which the females were almost always pale in color and their wings and legs not fully extended, indicating that they were pairing immediately after emergence.

Male.—Body 2.5 to 3 mm in length, ground color dark brown, not highly setigerous in general appearance; wing expanse 6.5 to 7 mm; wings slightly clouded. Eyes reniform, widely separated from each other on dorsal side, distinctly pubescent; distance between them greater than one-third of head width or slightly less than vertical length of eyes (12 : 16); frontoclypeus with one pair of conspicuous setæ on lateroproximal region and very rarely with two pairs; antennæ 9-segmented, with a few short verticils; scape without setæ; pedicel elongate, with about five verticils on distal part, thrice as long as width, paler on proximal half; first segment of flagellum with two or three short verticils; second segment with one verticil; third to fifth usually without verticils; sixth segment longer than each of preceding four segments, with about four verticils; distal segment fusiform, with four verticils on proximal region and two, short, apical setæ; antennal ratio about 0.5, varying from 0.4 to 0.6; each maxillary palpus with elongate ultimate segment and three moniliform proximal segments.

Pronotum setigerous only at side; scutum with a few short setæ along pseudosutural foveæ, these setæ arising from pale basal punctures; supra-alar setal group represented by three or four black setæ; scutellum brown, and its entire surface hairy. Hypopygium (Plate 2, fig. 8) very simple; ultimate tergum trilobate, slightly setigerous, with small setæ along caudal margin, with minute hyaline anal point; coxites elongate, large, beset

with small setæ; styles also with small setæ, each with four (3 to 5) minute black teeth and several hyaline spines at tip.

Legs entirely dark brown; fourth tarsal segment distinctly obcordate; claws simple, not serrate at tip, with about three long setæ at each base; one tibial spur on foreleg, two on each middle and hind leg; these spurs flattened and pubescent basally, bare and chitinized at tip; tibial comb of hind leg consisting of eighteen strong bristles; tarsus of each leg with minute spurs on each of the proximal three segments; the foreleg having the following proportional lengths of segments: 90 : 88 : 57 : 28 : 17.5 : 7 : 8; leg ratio about 0.64 to 0.65. Wings (Plate 1, fig. 5) broad, hyaline, clouded with gray on marginal area, without macrotrichia; main veins brown, with small setæ; costa not produced beyond end of R_{4+5}; R_1 and R_{4+5} more or less swollen distad, relative lengths between them about 10 : 17 or 10 : 19; R_{2+3} ending on costa, very close to tip of R_1; r-m very slightly curved, a little longer than three times first section of M_{3+4}; m-cu very short, equal in length to width. Halteres yellow.

Female.—Body 2.6 to 3 mm in length; wing expanse 5.2 to 7 mm. Main differences from the male as follows: Antennæ 8-segmented, with a few verticils; distal segment shorter than preceding four segments of flagellum taken together, with two short apical setæ and three verticils on proximal part; antennal ratio about 0.4; leg ratio of foreleg about 0.61, very rarely attaining 0.7; hypopygium without distinct long setæ; ultimate sternum dark, entirely pubescent, with large thickened lamellæ along caudal margin, with a small, V-shaped, caudal incision, without distinct setæ; ultimate tergum (Plate 2, fig. 11) membranous, slightly hairy at side, with a pair of small, blunt, setigerous projections on caudal margin near bases of cerci; cerci (Plate 1, fig. 6) short, somewhat rectangular, pale brown; spermathecæ two, elongate, ellipsoid, brown, without colored neck region.

Pupa.—Body 3.4 to 4 mm in length; exuviæ ochraceous, hyaline, entirely covered with minute dotlike spinules; dorsal spinous welts seven in both sexes, ventral spinous welts four in female and five in male. Vertex with strong paired setæ near antennal bases; base of antennal sheath distinctly projected cephalad, forming a characteristic tuberculous horn (Plate 3, fig. 22). Thorax with several small setæ, which are not distinct, excepting a pair of large setæ cephalad of respiratory organs; prothoracic respiratory organs small, filiform, flattened at middle, not pubescent (Plate 3, fig. 23). In both sexes one dorsal spinous

welt on each abdominal segment from second to eighth, in female one ventral welt on each segment from fourth to seventh, and in male on each segment from fourth to eighth, thus making the general characters of the present pupa different from those of *Diamesa* pupæ as described by Potthast,[3] in the absence of the ventral welt of third abdominal segment; spines of dorsal welts small, dark, solid, conical, not curved backwards; those of ventral welts smaller than those of dorsal welts; number of spines varying in different segments, individuals, and sexes from twelve to fifteen on dorsal welts and from twenty to twenty-four on ventral welts, excepting those of modified eighth segment; typical arrangement of abdominal setæ as follows: Five pairs of small setæ on each dorsum, four pairs of small setæ on each venter, and a minute and three large setæ on each lateral expansion; lateral expansion comparatively narrow, provided with a rudiment of spiracle on dorsocephalic region and a very small pointed projection on caudal margin; eighth segment provided with two large setæ on each small lateral expansion, two pairs of small setæ on each lateral side of dorsum and venter; in female dorsal welt consisting of only about ten spines, and ventral wall of this segment expanded caudad forming a large swollen part of genital sheath; in male ventral welt of eighth segment composed of eight or ten spines; ultimate segment somewhat semielliptical from dorsal aspect, its dorsal side almost flattened, without setæ; lateral and caudal margins flattened forming lamellalike edges; caudal margin deeply constricted at meson, provided with a pair of setal groups each consisting of three, long, brown setæ; in female sheaths of hypopygium small, bilobate, not extending beyond caudal margin of ninth segment (Plate 3, figs. 20 and 21); in male hypopygial sheaths large, deeply bilobate, extending caudad far beyond caudal margin of ninth segment, then strongly curving backward, as in the *Diamesa* group in general.

Habitat.—Japanese Alps, Japan.

Holotype.—Male; Tsurugisawa, Toyama Prefecture; October 16, 1928.

Allotopotype.—Female; October 16, 1928.

Paratypes.—Males and females; head, legs, wing, and hypopygium mounted in euparal; Tsurugisawa, Toyama Prefecture; October 16 and 17, 1928, and June 14, 1935; and Kakogawa, Nagano Prefecture, April 1, 1930.

[3] Arch. Hydrobiol., Suppl.-Bd. 2 (1915) 350–361.

Type specimens.—Alcoholic; deposited in the entomological laboratory of Kyoto Imperial University; collected by Mr. K. Imanishi.

This species is related to *Diamesa borealis* Coquillett and *D. culicoides* Heeger, but differs distinctly in the dark brown or brown maxillary palpi, antennæ, scutellum, and legs of the male and the longer crossvein r-m of both sexes from the former allied species and differs in the higher value of the antennal ratio of the male from the latter species. A female specimen collected by Mr. S. Hosono at Seki, Nigata Prefecture, March 31, 1927, is slightly different from the other specimens only in the short ultimate segment of the antennæ, the antennal ratio being reduced to 0.3. This may be an aberrant form of this species.

DIAMESA (DIAMESA) JAPONICA sp. nov.

This fly was captured on the snow in the Japanese Alps, 200 to 1,600 meters in altitude.

Male.—Body about 4.1 mm in length, ground color black; wing expanse about 7.2 mm. Eyes pubescent, reniform, widely separated from each other on dorsal side; frontoclypeus with only four pairs of long setæ on lateroproximal region; antennæ 9-segmented, not plumose, with only a few short verticils, 0.54 mm in total length; scape without setæ; pedicel equal in length to following three segments of flagellum together, entirely brown, with three verticils on distal region; ultimate segment of antenna elongate, cylindrical, longer than remaining segments of flagellum together, with two small apical setæ, with six long verticils on proximal region; antennal ratio about 0.81; remaining segments of flagellum moniliform; first and second segments each with one verticil; other intermediate segments without verticils; maxillary palpi dark brown, moniliform, distinctly 5-segmented (4 : 5 : 9 : 10 : 16); distal segment pale and cylindrical.

Thorax entirely black; pronotum slightly setigerous at side; scutum without median setæ; erect black setæ along the pseudosutural foveæ arising from dorsal black punctures; scutellum sparsely beset with long black setæ. Hypopygium (Plate 2, fig. 7) with very small setæ, darker than anterior segments; ultimate tergum with a large, membranous, anal point, very sparsely setigerous along caudal margin; coxites each with a dorsomesal membranous lobe which is hardly extended to the tip of anal

point; styles with minute setæ, each provided with an apical tooth.

Legs entirely black; fourth tarsal segments obcordate, shorter than fifth; all tarsi with apical spurs on each of proximal three segments; tibial spurs highly pubescent, small, far shorter than diameter of tibial end, each with a minute chitinized tip; tibial comb of hind leg consisting of about twenty-two strong bristles; proportional lengths of segments of foreleg 116 : 109 : 63 : 30 : 19 : 7 : 10; leg ratio about 0.57 to 0.58. Wings broad, brownish under transmitted light, hyaline, clouded on margin, especially on anal margin, without macrotrichia; main veins dark brown; costa slightly produced beyond end of R_{4+5}; R_1 and R_{4+5} more or less swollen distally, their relative lengths about 23 : 37; R_{2+3} ending very close to end of R_1; r-m angulate or strongly curved, about thrice as long as first section of M_{3+4}; m-cu slightly shorter than first section of M_{3+4}; Cu_1 curved at tip. Halteres yellowish white.

Female.—Generally similar to male; body about 4 mm in length; frontoclypeus more setigerous than in male, being provided with ten pairs of setæ; antennæ more setigerous, with long verticils, 8-segmented; antennal ratio about 0.4; pedicel equal in length to following two segments taken together, with five verticils on distal region; proximal two flagellar segments each provided with about five setæ; third with three; fourth and fifth each with two; sixth with about five proximal and two long distal verticils, fusiform, equal in length to preceding three segments taken together; maxillary palpi 5-segmented (2 : 4 : 8 : 7 : 13); leg ratio of foreleg 0.57 to 0.58; tibial comb of hind leg consisting of about twenty-three setæ; ultimate tergum dark brown, very slightly setigerous, not reduced into membrane; ultimate sternum dark, very slightly setigerous at side, with only three small setæ on either side; cerci (Plate 2, fig. 12) pale at base, somewhat triangular, projected ventrad; spermathecæ two, small, spherical, brown, with brown neck. Other structures closely resembling those of male.

Pupa.—Body about 4 mm in length; exuviæ ochraceous, slightly brown on head, thorax, and caudal end; abdominal spinous welts consisting of large brown spines arranged on prominent brown ridges. Head with a pair of large setæ on vertex near bases of antennæ; projections of antennal bases very small, indistinct. Thorax with six pairs of distinct setæ:

Two pairs on pronotal region, three near respiratory filaments, and one pair on middle of scutal region; these thoracic setæ more distinct in male than in female; respiratory organs (Plate 3, fig. 24) very much elongate, filiform, not distinctly flattened at base. Abdominal spinous welts arranged on following segments: In female seven dorsal welts on segments second to eighth, and five ventral welts on segments third to seventh, and in male seven dorsal welts as in female and six ventral welts on segments third to eighth; in both sexes first ventral welts (Plate 3, fig. 25) highly reduced, only spines of lateral part remaining, each welt consisting of several large and small spines, larger ones being five to seven on one welt in male and six to nine in female; spines of ventral welts smaller, more slender than those of dorsal and slightly curved cephalad (Plate 3, figs. 26 and 27); setal arrangement of abdominal segments as in *Diamesa alpina,* but lateral expansions better developed; welts of seventh and eighth segments of female more or less reduced than in preceding segments; dorsal welts of these two segments each provided with six large spines, and ventral welt of eighth segment completely wanting; venter of eighth segment of female swollen ventrocaudad, provided with a line of minute dotlike spinules on caudal margin instead of welt. Ultimate segment of both sexes broad, somewhat trapezoid from dorsal aspect, with broad marginal ridge, deeply constricted at caudomeson, provided with a pair of setal groups on each caudal margin; these setal groups comparatively widely separated from each other, each consisting of three, long, curved setæ; structures of genital sheath as in *Diamesa* in general. Structures of abdominal spinous welts closely resembling those of *Diamesa insignipes* Kieffer, but differing in the spines of one welt being less than ten.

Habitat.—Japanese Alps, Japan.

Holotype.—Male; hypopygium mounted in euparal; Kashima, Nagano Prefecture; June 1, 1928.

Allotype.—Female; head, legs, and hypopygium mounted in euparal; Sasagamine, Nigata Prefecture; March 27, 1932.

Paratypes.—Males and females; Kinebashi, Fukui Prefecture; February 10, 1930; and Hosono, Nagano Prefecture; March 17, 1935.

Type specimens.—Alcoholic; deposited in the entomological laboratory of Kyoto Imperial University; collected by Messrs. K. Imanishi and K. Tsutsui.

This species is related to *Diamesa borealis* Coquillett and *D. culicoides* Heeger, but distinctly separated from the above two allied species by the antennal structures and coloration of the body, as in *D. alpina* Tokunaga. Another allied species, *D. waltli* Meigen, has a comparatively high value of the antennal ratio, and the antennæ of the male are 14-segmented and plumose.

DIAMESA JAPONICA var. (No. 1).

This variety was captured on the spring snow at Seki and Sasagamine, Nigata Prefecture, and closely resembles the type; it differs only in the following structures: Value of leg ratio slightly larger, being 0.56 to 0.6; nonsetigerous, ultimate sternum; rounder cerci (Plate 2, fig. 13); and nonspherical, comma-shaped spermathecæ (Plate 2, fig. 17).

Specimens.—Females; Seki and Sasagamine, Nigata Prefecture; March 26 and April 2, 1928; collected by Messrs. K. Imanishi and S. Hosono.

DIAMESA (DIAMESA) ASTYLA sp. nov.

This peculiar fly was collected on snow at Tsurugisawa, Toyama Prefecture, 1,600 to 2,000 meters in altitude.

Male.—Body small, 3 mm in length, dark brown, slightly setigerous in general appearance; wing expanse about 5.7 mm. Head oval, 0.41 mm in width; eyes reniform, hairy, very widely separated from each other; distance between eyes about three-fifths as great as total width of head; frontoclypeus pubescent but without setæ; antennæ not plumose, sparsely beset with several short verticils, about 0.42 mm in total length, resembling female antennæ of this genus in general appearance; scape without setæ; pedicel thrice as long as its width, with four short verticils on distal region; distal segment of antenna large, fusiform, equal in length to pedicel, with two very short apical setæ and three verticils on proximal region; intermediate five flagellar segments moniliform, each slightly longer than width, without verticils or with one or two; proportional lengths of antennal segments as follows: 6 : 2.2 : 2 : 2 : 2.2 : 3 : 6 : 3; antennal ratio 0.35; maxillary palpi yellowish brown, moniliform, 0.33 mm in total length; proportional lengths of segments 2 : 2.5 : 5 : 4.5 : 6.8.

Thorax dark brown; pronotum slightly setigerous at side; scutum with several pale setigerous punctures along each pseudosutural fovea, without middorsal setæ; scutellum dark brown, highly setigerous.

Legs entirely brown; tibial spurs small, far shorter than diameter of tibial end, flattened and pubescent basally, each with a minute bare tip; tibial comb of the hind leg consisting of about seventeen setæ; each of the proximal three tarsal segments of each leg with a pair of minute spurs; fourth segment of the tarsus obcordate, shorter than fifth; segments of foreleg having the proportional lengths 78 : 71 : 50 : 22 : 14 : 5.5 : 7.5; leg ratio about 0.7.

Hypopygium (Plate 2, fig. 9) peculiar in the absence of movable styles, entirely dark, beset with small setæ; ultimate tergum, subdivided by a pair of dorsal chitinizations into a median, pyriform, setigerous plate and paired, lateral, setigerous, semichitinized halves; median tergite without anal point, slightly constricted longitudinally at caudomeson, with two or four hyaline setæ on its caudal margin; coxites large, elongate, each with a small thickened pubescent tubercle at tip instead of style; this tubercle with three hyaline setæ at side; proximomesal lobes of coxites pubescent but without setæ, usually hidden under ultimate tergum.

Wings without macrotrichia; clouded at margin; main veins dark brown; costa very slightly produced beyond end of R_{4+5}; R_1 and R_{4+5} slightly swollen distally; R_{2+3} ending near tip of R_1; r-m very slightly curved, thrice as long as first section of M_{3+4}; m-cu apparently wanting, M_{3+4} being directly deflected on Cu_1. Halteres yellowish white.

Habitat.—Japanese Alps, Japan.

Holotype.—Male mounted in euparal; Tsurugisawa, Toyama Prefecture; October 16, 1928; deposited in the entomological laboratory of Kyoto Imperial University; collected by Mr. K. Imanishi.

This species differs from all the other known species of *Diamesa* by the unique character of the hypopygium of the male.

DIAMESA (DIAMESA) TSUTSUII sp. nov.

This fly was collected from the snow surface where *D. japonica, Trichocera nivalis, Capnia nivalis,* and certain snow Collembola were also found (Plate 4, fig. 31).

Male.—Body about 4.5 mm in length, entirely dark brown. Eyes pubescent; frontoclypeus setigerous with short brown setæ; antennæ 9-segmented (5 : 4.5 : 2.5 : 3 : 3 : 3 : 2.2 : 2 : 9); flagellar segments each with several short setæ; ultimate segment constricted before tip, with a short apical seta; second

antennal segment comparatively short, pale brown at proximal half; antennal ratio about 0.5. Pronotum slightly setigerous at side; mesothoracic stripes indistinct. Halteres yellowish white. Wings as in *D. japonica;* costa slightly produced beyond end of R_{4+5}; R_1 and R_{4+5} somewhat swollen; r-m curved but less than in *D. japonica* and more than in *D. alpina,* ending on base of M_{1+2}. Relative lengths of segments of foreleg 93 : 108 : 62 : 30 : 20 : 8 : 10; proximal two tarsal segments of all legs each provided with apical spurs; leg ratio about 0.57. Penultimate abdominal tergum separated into paired, lateral, subtriangular, setigerous plates; ultimate tergum (text fig. 2, *a*) with a sharply pointed anal point and paired lateral plates.

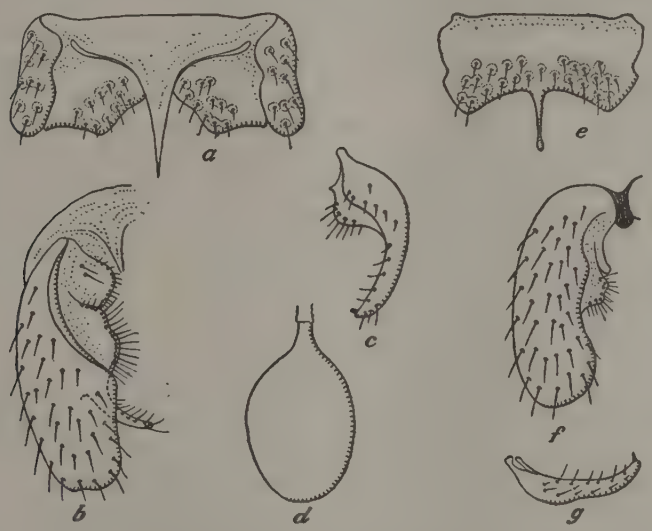


FIG. 2. *Diamesa tsutsuii* sp. nov. and *D. plumicornis* sp. nov.; *a* to *d*, *D. tsutsuii; a*, ultimate tergite; *b*, coxite, ventral aspect; *c*, style, lateral aspect; *d*, spermatheca; *e* to *g*, *D. plumicornis; e*, ultimate tergite; *f*, coxite, ventral aspect; *g*, style.

Coxites (text fig. 2, *b*) forming a large, ventral, genital chamber between thickened proximal ridges of coxites, each with three mesal lobes, of which the proximal is fringed with very small setæ and with two or three small setæ on ventral side, middle lobe large, fringed with long setæ and third lobe slender with small setæ on mesal side; two, proximal, flattened lobes forming dorsal wall of genital chamber. Style (text fig. 2, *d*) distinctly curved, with a triangular, basal projection on lateral side and a small apical spine.

Female.—Body length about 5.8 mm; antennæ 8-segmented (5 : 6 : 3.5 : 4 : 3.5 : 3.5 : 3 : 10); antennal ratio 0.42 (10 : 24); crossvein r-m ending at fork of M; proportional lengths of segments of foreleg 115 : 134 : 85 : 34 : 25 : 10 : 12; leg ratio about 0.63; penultimate abdominal tergum pale brown on caudal half; ultimate tergite subdivided into lateral halves by a narrow, median, transversal, membranous area; cerci pale brown, subtriangular; spermathecæ (text fig. 2, *d*) ovoid, with dark neck region. Other structures as in male.

Habitat.—Mountainous regions; Japan.

Holotype.—Male; Hosono (about 600 meters in altitude), Nagano Prefecture; March 17, 1935.

Allotopotype.—Female; March 17, 1935.

Type specimens.—Alcoholic; deposited in the entomological laboratory, Kyoto Imperial University; collected by Mr. K. Tsutsui.

This species is named in honor of the collector of this interesting midge. In general appearance the fly resembles *D. japonica,* but the structure of the male hypopygium is characteristic. The hypopygium is similar to that of *D. camptoneura* Kieffer, but the male antennæ are highly different in the absence of plumose hairs and the number of segments.

DIAMESA TSUTSUII (summer form ?).

This is slightly different from the type in the following characters: Body 4 to 6 mm in length; dark brown or brown in ground color; ultimate antennal segment with two, small, apical setæ; antennal ratio 0.47; leg ratio 0.68 to 0.8. Cerci (Plate 2, fig. 15) pale brown; spermathecæ as in Plate 2, fig. 19. Presumably these female specimens are the summer form.

Specimens.—Alcoholic females; Renge, Toyama Prefecture; August 15, 1931; and Kamikochi, Nagano Prefecture; July 17 to 21, 1932; deposited in the entomological laboratory of Kyoto Imperial University; collected by Mr. K. Imanishi and by Mr. Tokunaga.

DIAMESA (DIAMESA) PLUMICORNIS sp. nov.

This fly was found at Mount Hiei, Kyoto.

Male.—Body 5.5 mm in length, dark brown in ground color; abdomen brown, pale brown on the sternal side. Eyes and frontoclypeus setigerous. Antennæ 14-segmented, highly plumose, with dark hairs; each with a short apical seta; ultimate segment slightly swollen distally, with a papilliform projection at tip; an-

tennal ratio over 1 (33 : 31). Pronotum setigerous at side. Costa slightly produced beyond end of R_{4+5}; R_1 slightly swollen; cross-vein r-m ended on M_{1+2}, almost straight. Halteres white. Proportional lengths of segments of foreleg 82 : 96 : 66 : 31 : 20 : 7 : 8; leg ratio about 0.69; proximal two tarsal segments of each leg with apical spurs. Ultimate tergum (text fig. 2, *e*) uniformly thickened, with dark, slender, anal point, which is not sharply pointed; coxites (text fig. 2, *f*) with a setigerous mesal lobe and a minute, nonsetigerous, fingerlike lobe; style (text fig. 2, *g*) very slender, slightly curved, with a minute apical spine.

Habitat.—Kyoto, Japan.

Holotype.—Alcoholic male; Mount Hiei, Kyoto; March 17, 1935; deposited in the entomological laboratory of Kyoto Imperial University; collected by M. Tokunaga.

This fly is the only known Japanese species of the subgenus *Diamesa* that is provided with plumose antennæ. In general appearance it somewhat resembles *D. waltli* Meigen and *D. hamaticornis* Kieffer, but they are distinctly different from each other in the structure of the hypopygium and in the value of the antennal ratio.

DIAMESA sp. (No. 1).

This fly was captured at light at Renge, Japanese Alps, Toyama Prefecture, altitude, about 2,000 meters, in the summer season.

Female.—Body comparatively large, 4.5 mm in length, black in ground color. Head dark brown; eyes hairy; reniform, widely separated from each other; frontoclypeus setigerous with about twelve setæ; antennæ 8-segmented, setigerous; three verticils on scape, six on pedicel, five on first flagellar segment, four on second, three on third, four on fourth, two on fifth, and two on ultimate in addition to two, short, apical setæ; ultimate segment of antenna slightly shorter than preceding three segments together (50 : 54); antennal ratio about 0.73; maxillary palpi elongate, cylindrical, far longer than the antennæ; proportional lengths of segments 7 : 11 : 10 : 17. Pronotum setigerous at side; scutum slightly pruinose, without middorsal setæ; supra-alar setæ eight. Leg ratio of foreleg 0.7 to 0.8; relative lengths of foreleg segments 105 : 127 : 90 : 39 : 23 : 8 : 9; fourth tarsal segments lobate, shorter than fifth; tibial spurs large, not flattened basally, hardly as long as diameter of tibial end; tibial comb of hind leg consisting of about twelve setæ; each of the two proximal tarsal segments of all legs provided with a pair

of small spurs. Hypopygium setigerous; ultimate tergum subdivided by a median membrane into lateral, setigerous tergites; ultimate sternum dark, setigerous at side, with a shallow, caudal incision; cerci (Plate 2, fig. 14) projecting ventrad, pale at base; spermathecæ (Plate 2, fig. 18) two, brown, each with brown neck region. Wings without macrotrichia; costa slightly produced beyond end of R_{4+5}; R_1 and R_{4+5} more or less swollen at tip; R_{2+3} ending close to end of R_1; r-m distinctly curved; m-cu very short. Halteres yellow.

Habitat.—Japanese Alps, Japan.

Specimen.—Female; legs mounted in euparal; Renge, Toyama Prefecture; August 15, 1931; deposited in the entomological laboratory of Kyoto Imperial University; collected by Mr. K. Imanishi.

DIAMESA sp. (No. 2).

This female specimen was collected on snow at Seki, Nigata Prefecture, and has the following distinctive characters: Body length about 4.3 mm; ground color dark brown; eyes finely pubescent with minute, brown, dotlike hairs; frontoclypeus highly setigerous; antennæ 8-segmented, setigerous; scape with four setæ; ultimate segment of antenna fusiform, longer than preceding three segments together (12 : 10), with one proximal verticil and two apical setæ; antennal ratio about 0.52; segments of maxillary palpi 3 : 7 : 11 : 12 : 17 in relative lengths; wings with slightly curved r-m; halteres yellowish white; proportional lengths of segments of foreleg 99 : 122 : 84 : 40 : 24 : 9 : 16; leg ratio about 0.7; tibial comb of hind leg composed of twenty-one setæ; hypopygium as in *Diamesa* sp. (No. 1).

Habitat.—Nigata, Japan.

Specimen.—Female, head, legs, and wings mounted in euparal; Seki, Nigata Prefecture; March 23, 1928; deposited in the entomological laboratory of Kyoto Imperial University; collected by Mr. S. Hosono.

This female is closely related to *Diamesa* sp. (No. 1), but differs distinctly in the following points: Very short pubescence of eyes; more setigerous frontoclypeus, less-produced costa, slightly curved r-m, and the value of the antennal ratio.

ILLUSTRATIONS

Plate 1

Fig. 1. *Syndiamesa lanceolata* sp. nov., male hypopygium, dorsal aspect.
2. *Prodiamesa bathyphila* Kieffer, male hypopygium, ventral aspect.
3. *Syndiamesa kashimæ* sp. nov., male hypopygium, dorsal aspect.
4. *Syndiamesa montana* sp. nov., male hypopygium, dorsal aspect.
5. *Diamesa alpina* sp. nov., male wing; *al*, anal lobe; *f*, first section of M_{3+4}; *m-cu*, mediocubital crossvein; *r-m*, radiomedial crossvein; *sc*, squama.
6. *Diamesa alpina* sp. nov., female cercus, lateral aspect.

Plate 2

Fig. 7. *Diamesa japonica* sp. nov., male hypopygium, dorsal aspect; *ad*, appendage of coxite; *ap*, anal point.
8. *Diamesa alpina* sp. nov., male hypopygium, dorsal aspect.
9. *Diamesa astyla* sp. nov., male hypopygium, dorsal aspect.
10. *Diamesa nigatana* sp. nov., male hypopygium, dorsal aspect.
11. *Diamesa alpina* sp. nov., ultimate tergum of female, dorsal aspect.
12. *Diamesa japonica* sp. nov., female cercus, lateral aspect.
13. *Diamesa japonica* var. (No. 1), female cercus, lateral aspect.
14. *Diamesa* sp. (No. 1), female cercus, lateral aspect.
15. *Diamesa tsutsuii* (summer form ?), female cercus, lateral aspect.
16. *Diamesa japonica* sp. nov., spermatheca.
17. *Diamesa japonica* var. (No. 1), spermatheca.
18. *Diamesa* sp. (No. 1), spermatheca.
19. *Diamesa tsutsuii* (summer form ?), spermatheca.

Plate 3

Fig. 20. *Diamesa alpina* sp. nov., female pupa, three caudal segments, dorsal aspect.
21. *Diamesa alpina* sp. nov., female pupa, three caudal segments, ventral aspect.
22. *Diamesa alpina* sp. nov., pupa, tubercle of the antennal base.
23. *Diamesa alpina* sp. nov., pupa, prothoracic respiratory organ.
24. *Diamesa japonica* sp. nov., pupa, prothoracic respiratory organ.
25. *Diamesa japonica* sp. nov., pupa, ventral spinous welt of third abdominal segment, caudal aspect.
26. *Diamesa japonica* sp. nov., pupa, ventral spinous welt of fifth abdominal segment, caudal aspect.
27. *Diamesa japonica* sp. nov., pupa, dorsal spinous welt of fifth abdominal segment, caudal aspect.
28. *Syndiamesa montana* sp. nov., male pupa, ultimate segment, dorsal aspect.

FIG. 29. *Syndiamesa montana* sp. nov., male pupa, branched seta of lateral expansion of eighth abdominal segment.

PLATE 4

FIG. 30. Tsurugisawa, Japanese Alps, spring season. *Syndiamesa montana, Diamesa alpina,* and *D. astyla* were found on the snow in company with *Capnia nivalis* Ueno.

31. Hosono, Nagano Prefecture, spring snow. *Diamesa japonica, D. tsutsuii, Trichocera nivalis, Capnia nivalis, Capnia* sp., and snow Collembola were found on the snow surface of the forest zone.

32. Egg cords of *Diamesa alpina* adhering to a twig found at margin of a torrent.

TEXT FIGURES

FIG. 1. *Prodiamesa brevitarsis* sp. nov.; *a,* male hypopygium, dorsal aspect; *b,* male antenna.

2. *Diamesa tsutsuii* sp. nov. and *D. plumicornis* sp. nov.; *a* to *d, D. tsutsuii; a,* ultimate tergite; *b,* coxite, ventral aspect; *c,* style, lateral aspect; *d,* spermatheca; *e* to *g, D. plumicornis; e,* ultimate tergite; *f,* coxite, ventral aspect; *g,* style.

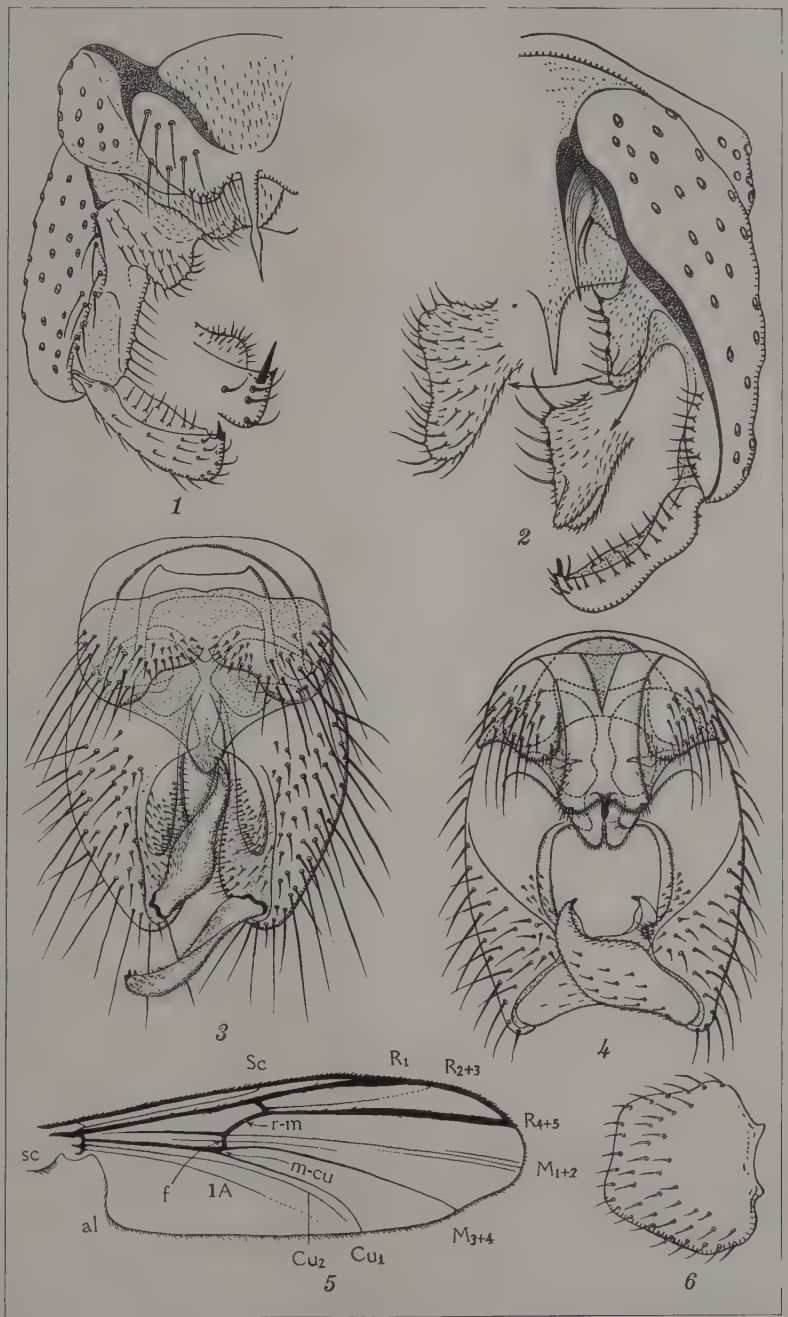


PLATE 1.

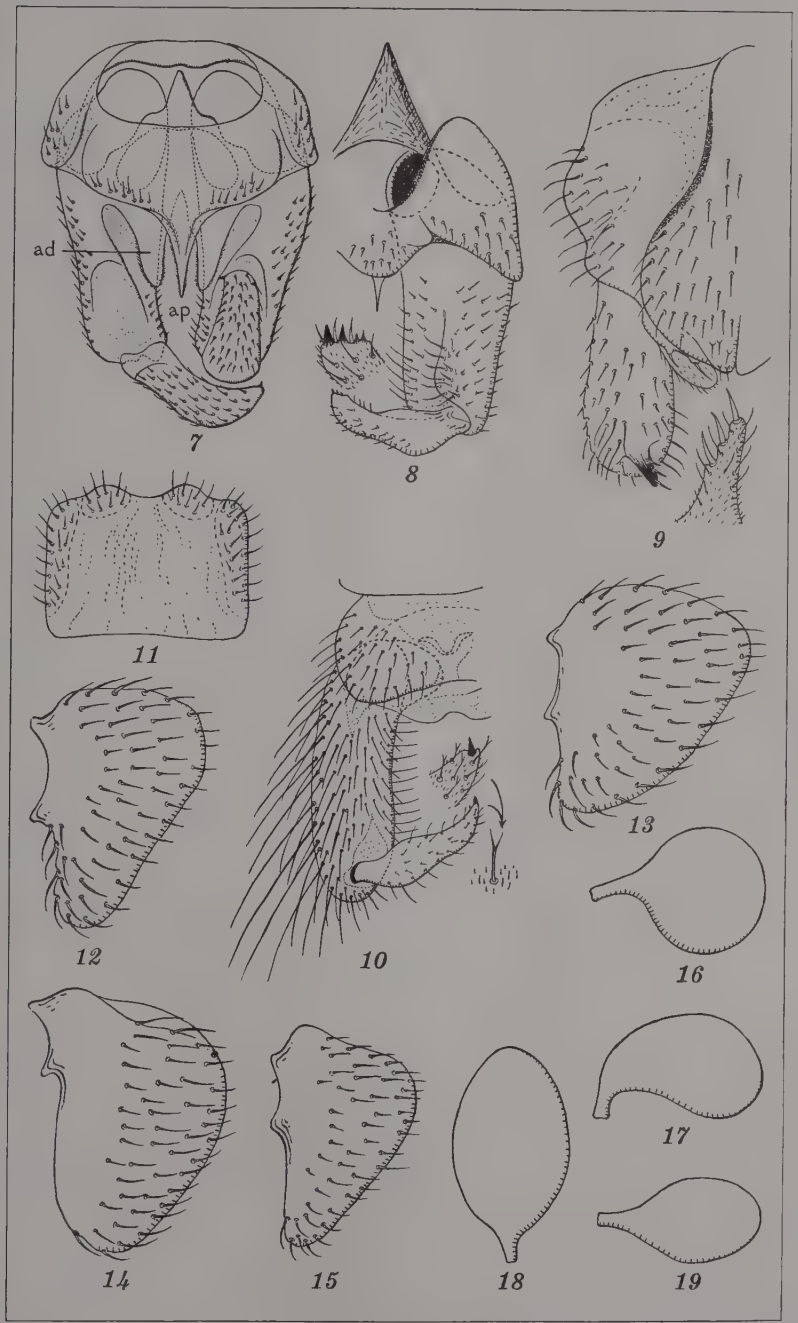


PLATE 2.

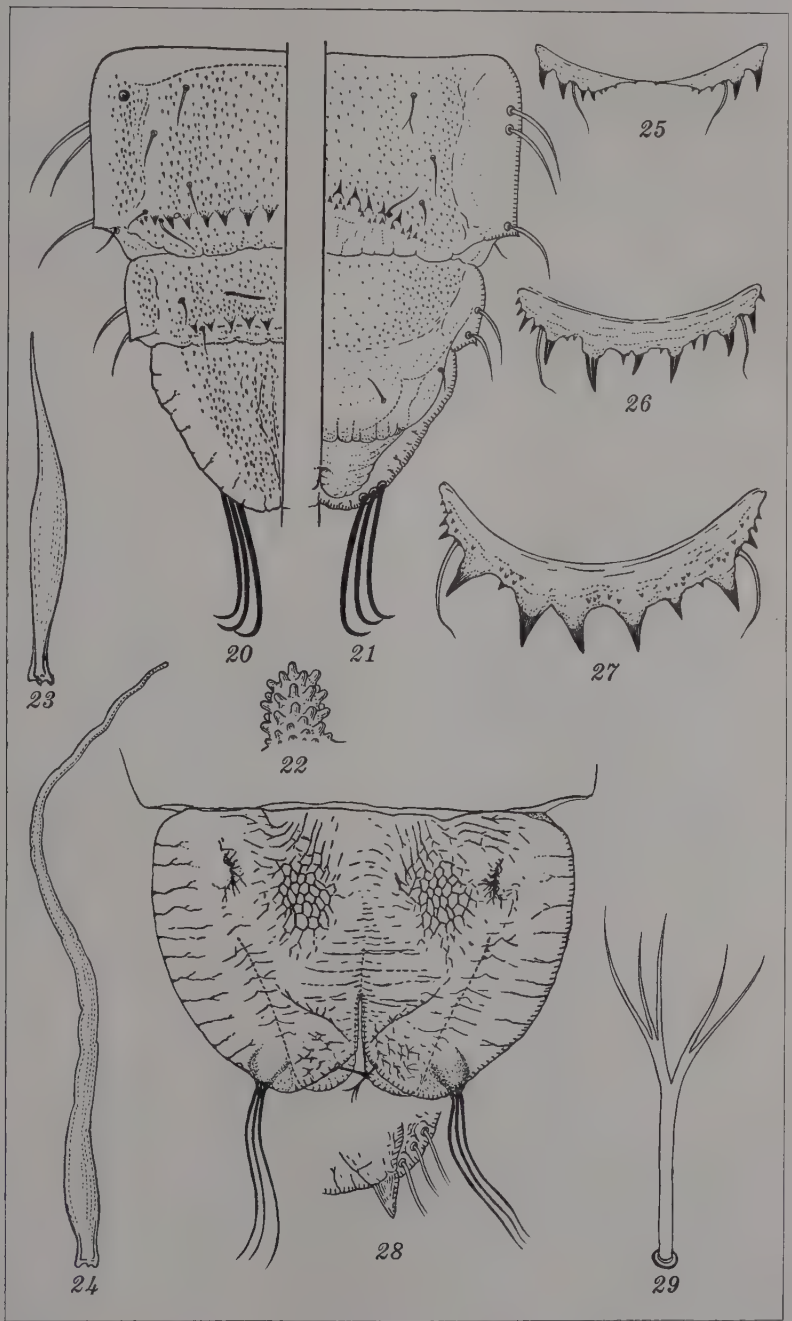
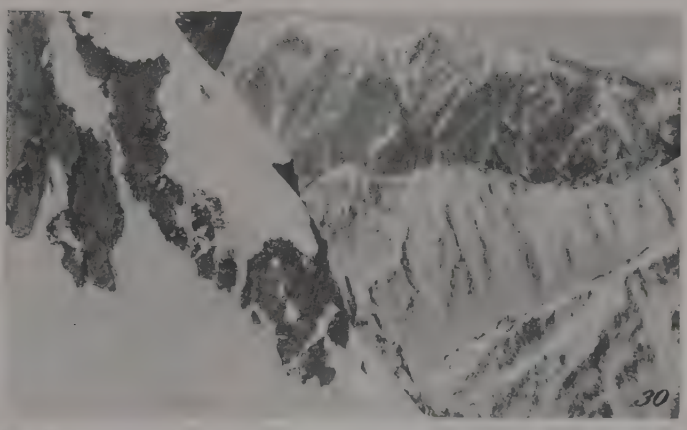


PLATE 3.



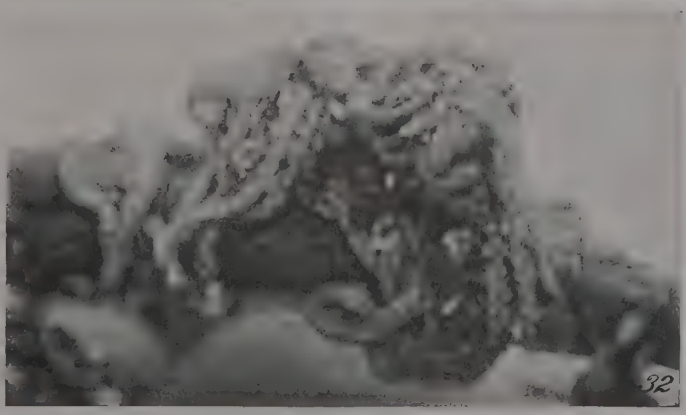


PLATE 4.

PHILIPPINE PSYCHODIDÆ (DIPTERA), I PSYCHODINÆ [1]

By F. DEL ROSARIO

Of the National Museum Division, Bureau of Science, Manila

TWO PLATES AND FOUR TEXT FIGURES

The subfamily Psychodinæ comprises a group of nonblood-sucking moth flies belonging to the large family Psychodidæ. They are generally of small size, usually over 1 millimeter long, rarely attaining 3 millimeters. They can be distinguished from members of other subfamilies, especially Phlebotominæ, by the wings being folded rooflike over the abdomen, like tiny moths, and by the fact that the first forking of the second vein is near the base, instead of nearer the middle, of the wing.

The subfamily Psychodinæ, which contains at present a number of genera, is practically unknown in the Philippines. So far as I can ascertain, no species of this subfamily has hitherto been recorded from this country. While working the psychodid collection of the Bureau of Science, I discovered one specimen belonging to the genus *Psychoda*. This is evidently a distinct species and is apparently new to science. Two specimens collected by me at Baguio, Mountain Province, Luzon, may be treated here as two distinct species. From collections made in Alabang and San Pedro Makati, Rizal Province, and Manila, all in central Luzon, four species seem to be very distinct and are here described as new species.

The psychodid flies discussed, therefore, represent seven new species of the genus *Psychoda*. Three other species of the same genus are also included. The types of the new species described in this paper will be deposited in the Bureau of Science entomological collection.

Inasmuch as the literature dealing with the classification of the Psychodinæ shows some confusion in the status of certain genera, and since the morphology of the entire subfamily is at best but imperfectly known, I deemed it advisable to describe

[1] I am under obligation to Dr. L. B. Uichanco, professor and head of the Entomology Department, College of Agriculture, University of the Philippines, for reading the manuscript.

the external morphology of the adults of the genus *Psychoda*. These accounts are drawn from materials in the collection of the United States National Museum, from the Bureau of Science entomological collection, and from the author's personal collection, and summarize the important characters common to all the species I have examined.

PSYCHODIDÆ

PSYCHODINÆ

Genus PSYCHODA Latreille

Tipula, pt. LINNÆUS, Syst. Nat. (1859).
Psychoda LATREILLE, Precis d. caract. gen. d. Ins. (1796).
Trichoptera MEIGEN, Illiger's Mag. Ins. 2 (1803).

Very close to *Pericoma*, from which it may be distinguished by the rather elongate antennæ having fourteen to sixteen segments, the segments of the flagellum, except segment 13, possessing a basal spherical node and a long internode, the last three terminal segments of reduced size and may or may not be intimately united with each other.

Genotype.—*Tipula phalænoides* Linnæus.

EXTERNAL MORPHOLOGY

ADULTS

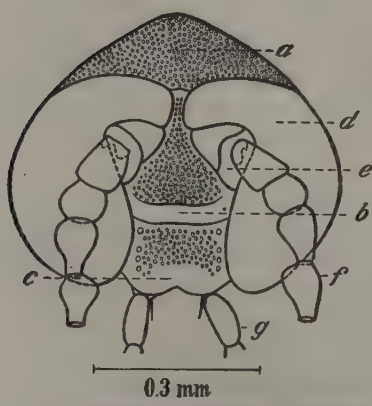


FIG. 1. *Psychoda albipunctata* Williston, female, head in cephalic aspect; *a*, occiput; *b*, frons; *c*, clypeus; *d*, compound eye; *e*, antennal fossa; *f*, antenna; *g*, maxillary palpus.

Occiput in both sexes usually covered with numerous, stout, forwardly projecting hairs. Eyes at frons separated by a distance varying from a fine line to about four times the diameter of one facet. Frons and clypeus together nearly triangular, with thick hairs (text fig. 1).

Proboscis about one-half as long as the head in both sexes. The mouth parts of the male are shown in text fig. 2. Tip of labium, or paraglossæ, may or may not be definitely enlarged with from four to six long or short teeth at the tip and with two to six long spines on the outer surface; those with enlarged lobe may not have any spine-

like teeth on its inner surface or may have as many as eight; the outer margin with four to twenty or more long and short spines. The remaining mouth parts are slightly chitinized and distally bear long and several short fine hairs. The labrum is unarmed and distally narrows to an almost acute apex bearing minute hairs. Mandible wanting. Hypopharynx well developed, strongly chitinized; distally narrowing to a pointed apex, bearing minute hairs. Maxillæ represented by a free, rodlike structure, the stipite, which is close to the hypopharynx and almost touching the anterior arm of the tentorium, and a feathery galea, which is nearly as long as the stipite. Cardines apparently wanting. Palpi in both sexes composed of four segments, the first segment generally the shortest. Sometimes the first three basal segments are of unequal length and the fourth only slightly longer. In some species the third is notably longer than the second. As a general rule, the terminal segment tapers and ends in a bluntly rounded apex bearing three to five or more long spines. In certain species the first and third segments are expanded near the middle or anteriorly and contain a deep pit or sensory cup; the inner surface of this pit bears a number of minute modified hairs shaped like a drum-stick.

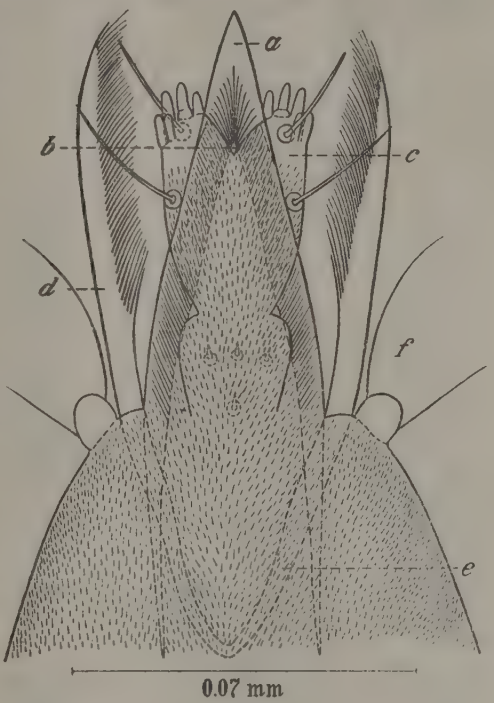


FIG. 2. *Psychoda makati* sp. nov., mouth parts, dorsal aspect; *a*, labrum; *b*, hypopharynx; *c*, labium; *d*, galea; *e*, stipite; *f*, palpus.

Antennæ composed of fourteen to sixteen segments, all the segments distinctly separated from each other. Segment 1 subcylindrical, longer than broad, with stout hairlike spines. Seg-

ment 2 (torus) globular, also provided with stout spinelike hairs arising at about the middle region of the node. Segments of the flagellum, excepting segment 13, with a basal spherical node and with a neck or internode which is usually as long as the node, often longer and sometimes shorter, especially in the female. Segment 13 generally without an internode but sometimes with a very short elongation of a rather insignificant length. The segments following segment 13 are quite diminutive and may be distinctly separated from each other or they may be partly or completely united. Hairs on these segments spinelike. Each segment of the flagellum, except the last three terminal, bears a whorl of five or six verticils of bristlelike hairs, usually recurved and pointing toward the node of each succeeding segment. Long and short sensory spines, or "ascoids," on segments 3 to 13; they are always two to the segment and are diametrically opposed in their point of insertion in the node. These sensory spines are of various shapes (text fig. 3), the usual form being a 3-branched spine, the two anterior branches projecting towards the internode and a posterior branch towards the node. In the female antennæ of *Ps. nigra* and *Ps. autumnalis* each sensory spine of the node is composed of five or more

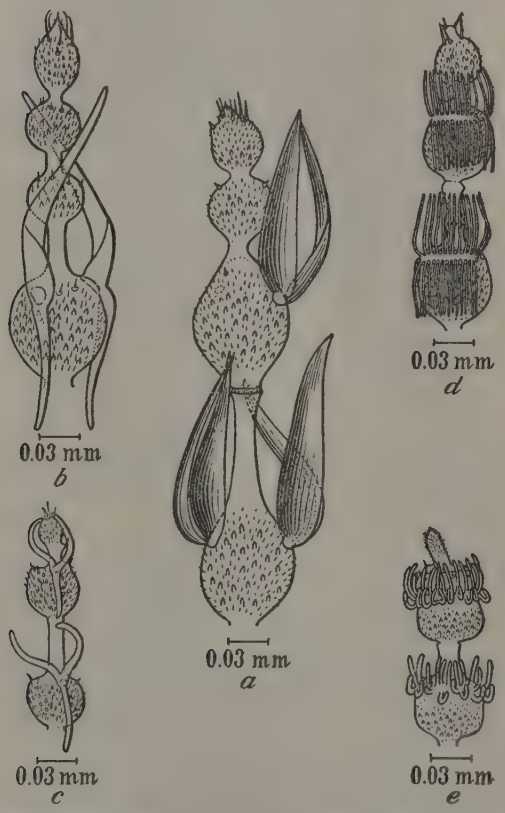


FIG. 3. *Psychoda*, five species, showing sensory spines at tip of antennæ of males; *a*, *Ps. olympia* Kincaid; *b*, *Ps. bicolor* Banks; *c*, *Ps. severini* Tonnoir. *d*, *Ps. snowhilli* del Rosario; *e*, *Ps. nigra* Banks.

fingerlike branches; these sensory spines do not surround the node as found in the male antennæ of the same species.

Thorax strongly arched and very slightly projecting over the head. Dorsum clothed with both short and long erect hairs. Scutellum thickly set with long straight hairs, which are grayish or whitish in many species, some with short hairs. Pleura usually fuscous brown or pale brown, in some species with short hairs.

Wings usually ovate or lanceolate, in many species gray with darker areas along the anterior margin or fringe. They are usually twice as long as wide, although in the male of some species the wings are a little less than twice as long as wide. Surface of the wings entirely covered with upright hairs, which may be either gray or white or both, longer recumbent hairs also present, especially along the anterior and posterior margin. Wings may show a mottled appearance as a result of the alternate black and white patches of erect hairs on the upper surface. In one species, *Ps. autumnalis,* a denuded wing is conspicuous for the presence of clear pigmented areas on the veins, which give the wing a distinctly mottled appearance. Venation as shown in Plate 1. Sc either short or long, the usual length being nearly that of the horn or semichitinous pad above it at the base of the anterior margin of the wing, apparently not ending at C. Fork R_2R_3 in most species before the level of fork M_1M_2, with four exceptions, or slightly behind in two species. In *Ps. nigra, superba, superba* var. *conspicua,* and *snowhilli* fork R_2R_3 is distinctly on the level with fork M_1M_2, while in *Ps. autumnalis* and *Ps. quadripunctata* fork R_2R_3 is found notably behind the level of fork M_1M_2. Bifurcation of R_2 and R_3 and M_1 and M_2 not always complete, as the origin of R_3 or M_2 is sometimes loose. This condition may be seen in *Ps. pusilla, uniformis,* and *tridactila* and in *bicolor* (only that of fork M_1M_2).

Legs generally pale brown, often with dark gray hairs. Femora of forelegs strongly developed, sides in posterior view with some rows of bristles; pile on the outer surface usually gray, in some species, as in *Ps. autumnalis,* with scattered whitish hairs on the basal half of the anterior surface; apically with a narrow white band. Fore tibiæ generally pale brown, often fuscous, seldom with white band, except in *autumnalis.* First tarsal segment the longest, almost twice as long as the second, second to fourth tarsal segments decreasing in length progressively, fifth segment being as long as second or slightly

longer. Bristles on apical end of first four tarsal segments of all legs spinelike. Claws of all legs equal.

Abdomen composed of nine segments, dorsum pale brown or brown, lateral margins usually lighter. Each abdominal segment thickly clothed dorsally with a ringlike series of erect, mingled, white and gray hairs. Abdomen of male appears to be slenderer than that of female; hypopygium very conspicuous.

Hypopygium of male (text fig. 4) very prominent and easily distinguishable by the presence of relatively large inferior appendages. Like those of the Culicidæ, hypopygium inverted after hatching so that ninth tergite is usually longer than the eighth; sternite narrowed to a small chitinized strip, which is somewhat arched, tergite extending posteriorly, slightly arched to fit the elongated inferior appendages. The whole segment well clothed dorsally with strong hairs, most thickly set on distal half and at its posterior margin. Anus at posterior end of ninth segment; anal flap, which is well covered with short, fine hairs, appearing to be intimately connected with anus. Inferior appendages well developed, generally longer than ninth segment, often slender, curving ventrally, swollen slightly near the base, and gradually tapering toward an acute point in many species; terminal spinules clavate, frequently less than basal portion of the segment, number varying from 1 to 15; clothed at its base with longer and shorter hairs intermixed with a few minute hairs. Superior appendages of two segments the length of which varies according to species; basal segment large, swollen near the base; proximal segment about as long as the basal, slender, tapering to an acute point, with short and weak spines above and below. Ædeagus varying in shape and size in different species, well chitinized and composed of from one to five parts, usually two.

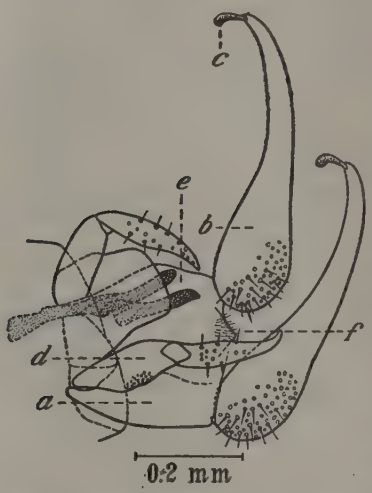


FIG. 4. *Psychoda alternata* Say, male hypopygium, dorsal view; *a*, ninth tergite; *b*, inferior appendage; *c*, terminal spinule; *d*, superior appendage; *e*, ædeagus; *f*, anal flap.

Ventral plate of female in many species terminating posteriorly in two finely pubescent lobes with a slight emargination between; densely clothed with black or gray hairs, depending on the species. Ovipositor consisting of two bladelike pieces, closely applied, about twice as wide as ventral plate, pointed, inner surface provided with a single row of short, hairlike spines.

PSYCHODA ALTERNATA Say.

Psychoda alternata SAY, Long's Exp. St. Peter's River App. (1823) 358.

In view of the detailed descriptions given in a previous article,[2] only the following characters need be noted.

The wings with a mottled appearance. Tips of several veins of wing with small patches of black or dark brown hairs. Outer surface of tip of labium with three labial spines. Antennæ of fifteen segments, the sensory spines on the node with two, well-developed, anterior branches and one posterior branch. Ædeagus consisting of two unequal parts; one shorter piece with an evenly pointed tip intimately attached to the latter.

Male.—Length of body, 1.5 millimeters; wing, 1.71; antenna, 1.32; width of head, 0.57; wing, 0.73.

Apparently a cosmopolitan species. It occurs throughout the United States, where it is most commonly found breeding on the bacterial film of the sprinkling filter beds used in the purification of sewage.

This is the first record of this species in the Philippine Islands. Several specimens, both males and females, were caught in a ditch along side of the street.

LUZON, Manila, July, 1935 (*G. Bellosillo*); *19355*, Bureau of Science entomological collection.

PSYCHODA ALBIPUNCTATA Williston.

Psychoda albipunctata WILLISTON, Ent. News 55 (1893) 113.

A revised description of this species has been given in a former paper (del Rosario, 1935) and need not be repeated here.

Male.—Length of body, 2.23 millimeters; wing, 2.60; antenna, 0.13; width of head, 0.7; wing, 1.28.

Like *Ps. alternata* this species is very wide-spread, but hitherto not recorded from the Philippines. Several specimens of all stages were collected by the author on standing water in

[2] Del Rosario, F., Philip. Journ. Sci. 59 (1936) 85–148.

the basement of the Bureau of Science building. They were breeding together with *Culex quinquefasciatus*.

LUZON, Manila, February, 1935 (*F. del Rosario*); *18863*, Bureau of Science entomological collection.

PSYCHODA PHALÆNOIDES (Linnæus, 1758).

Psychoda phalænoides (Linnæus, 1758) TONNOIR, Ann. Soc. Ent. Belgique 62 (1922) 67–68.

Male.—Length of body, 1.61 millimeters; wing, 1.59; antenna, 0.97; width of head, 0.35; wing, 0.63.

The specimens examined were collected from banana stalks together with *Ps. musæ* sp. nov. and *Ps. alabangensis* sp. nov. In America and Europe *Ps. phalænoides* has been reported breeding on the sprinkling filter beds of sewage plants together with *Ps. alternata*.

LUZON, Rizal Province, Alabang, July 10, 1935 (*F. del Rosario*), 7 males and 1 female; *19357*, Bureau of Science entomological collection.

PSYCHODA BAGUIOENSIS sp. nov. Plate 1, figs. 1 to 4.

Male.—Occiput black, borders lighter, covered with long, straight, yellowish hairs. Eyes confluent at frons. Frons and clypeus together triangular, dark brown, with yellowish hairs directed towards the proboscis, hairs at center of triangle thickly set and stout. Proboscis short, about as long as the second palpal segment, clothed with yellowish hairs. Tip of labium, or paraglossæ, expanded into a bulblike structure, each lobe possessing one short tooth on inner surface; outer surface of the lobe with about seven long spines and covered with fine, minute microtrichia. Palpi with yellowish hairs, slightly longer than width of the head; segment 1 shortest; segment 3 about as long as 2 or 4, but wider than any of the three segments; palpal segments in the proportion of 4 : 5 : 6 : 7. Nodes of antennæ black; hairs grayish except for the two segments before the last two, which are grayish white; 16-segmented; segment 1 small, about as long as wide; 2 a little smaller than 1 but wider than long; 3 to 12 flask-shaped, basal nodes about the same length as adjacent slender internode, over twice as wide as diameter of internodes; 13 like 12 but without any definite internode; 14, 15, and 16 small, of about the same size, somewhat ovoid, distinctly separated from each other by a very short neck; 16 bearing a short stout spine at apex. Sensory spines on segments 3 to 13 similar to those of *Ps. olympia* in shape and structure, but

differing in having only one spine on each side of the node. *Psychoda olympia* has two leaflike sensory spines on each side of the node.

Dorsum of thorax black with grayish white hairs on anterior margin; four batches of erect black hairs arranged in a band on posterior margin a little above costa near attachment of wing; an area clothed with silvery white hairs immediately behind. Sides of thorax brown, apparently bare. Wings somewhat ovate, over twice as long as wide, dark gray, with brownish tints; small white patches at tips of all longitudinal veins, starting tip of R_1; on R_1, beside the small white patch at its tip are two black spots, the one nearer the base of the wing composed of erect black hairs, minute white spots forming a whitish band running from tip of R_2 across to tip of M_3. Sc ending on level of origin of R_1. Origin of R_2R_3 on R_4 before level of r-m, fork R_2R_3 beyond middle of wing. Fork M_1M_2 closer to fork R_2R_3 than to r-m. Cell C and Sc darker and highly pigmented. Knob of halteres gray, stem yellowish. Legs black with grayish hairs. Tibiæ of all legs with several long erect hairs, which appear white under reflected light.

Abdomen dark brown dorsally, blackish beneath, the incisures pale brown to bronze; dorsum wholly covered with long dark gray hairs; base of venter with grayish brown hairs, toward tip with dark gray hairs; lateral margin either with pure dark gray or a mixture of dark gray and grayish brown hairs. Hypopygium concolorous with dorsum of abdomen, with uniformly dark gray hairs. Ninth tergite about as long as wide. Inferior appendages similar to those of *Ps. helicis*, stout, about as long as ninth tergite; entire surface covered with long and short spines; apex bearing two long spinules over half the length of the appendages. Basal segment of superior appendages broad, strongly developed; inner margin with one long spine; second segment clawlike, apex bearing a single, terminal, hairlike spine. Ædeagus consisting of three pointed parts, the median piece longest, the two side pieces strongly opposed against each other.

Length of body, 1.2 millimeters; wing, 1.31; antenna, 1.25; width of head, 0.34; wing, 0.56.

LUZON, Mountain Province, Baguio, February 10, 1935 (*F. del Rosario*), 1 specimen, male; *19357*, Bureau of Science entomological collection.

This species is distinguished by the antennæ, which have grayish white hairs on the two segments before the last two,

by the peculiar shape of the sensory spines, by the mottled appearance of the wings, by the black band on the dorsum of the thorax, and the form of the hypopygial appendages.

PSYCHODA ZIGZAGENSIS sp. nov. Plate 1, figs. 5 to 7.

Female.—Occiput black, clothed with dark gray hairs. Eyes at frons separated by a distance equal to a little over diameter of one facet. Frons and clypeus together triangular, black, covered with thick brushlike hairs, which are more or less recumbent. Proboscis golden with grayish hairs, over one and one-half times as long as first palpal segment. Tip of labium with three long teeth and one short tooth on each side of the lobe; outer surface with one, long, labial spine. Palpi dark gray, covered with grayish pruinescence; longer than width of the head; palpal segments nearly of equal length, apical segment bearing two spinelike hairs at tip; proportion of palpal segments 5 : 4 : 4 : 5. Nodes of antennæ dark gray; hairs grayish; 16-segmented; segment 1 the longest, much longer than wide; 2 globular; 3 to 13 flask-shaped, node of each segment nearly three times diameter of internode; 13 without internode and intimately connected with the three terminal segments, which are ovoid; 16 smallest. Sensory spines on segments 3 to 13 of the usual type, having two slender anterior branches and one posterior branch.

Dorsum of thorax pale yellow with erect yellowish hairs; pleura pale yellow with a few, scattered, yellowish hairs. Wings lanceolate, over twice as long as wide; pale yellow with yellowish hairs all over the veins; fringes slightly smoky. Sc about one and one-half times as long as the semichitinous pad above it, ending at about the same level as the origin of R_1. Origin of R_2R_3 on R_4 distinctly before the level of r-m, the fork at about the middle of wing. Fork M_1M_2 closer to fork R_2R_3 than to r-m. Halteres gray with yellowish hairs. Femora of forelegs dull brown with gray hairs; tibiæ and tarsal segments covered with black hairs, except claws, which are surrounded by a few, short, whitish hairs. Femora of middle and hind legs light brown, with light gray hairs; tibiæ and tarsi slightly darker under reflected light.

Abdomen dull brown, thickly clothed with yellowish hairs; genitalia covered with bristlelike yellowish to golden hairs. Ventral plate about twice as long as broad; posterior lobes distinctly separated by a deep notch; outer surface of lobes with

long bristles. Ovipositor about three times the width of the ventral plate, distinctly curved and pointed.

Length of body, 1.25 millimeters; wing, 1.34; antenna, 0.72; width of head, 0.34; wing, 0.51.

LUZON, Mountain Province, Baguio, February 10, 1935 (*F. del Rosario*); *19358*, Bureau of Science entomological collection.

This species may be distinguished from all other known forms by the single labial spine at the tip of the labium. It is the only one I have seen possessing this character, and none of the others have the tibiæ and tarsal segments of the forelegs wholly black.

PSYCHODA BANKSI sp. nov. Plate 1, figs. 8 to 12.

Male.—Occiput grayish brown with whitish hairs. Eyes at frons separated by a fine line. Frons slightly arched, light brown, covered with thick, recumbent, grayish hairs. Clypeus rather broad, less hairy than frons, hairs light brown. Proboscis short, light brown, yellowish pruinose. Tip of labium not well expanded into a bulblike structure, each lobe with two, rather long, slender teeth at margin and two short teeth on inner surface just below the two long teeth; outer surface with one short and two long labial spines; entire surface of labium covered with very short microtrichia. Palpi yellowish with gray hairs, about twice as long as proboscis; fourth segment longest, with three spines at extreme end; palpal segments in the proportion of 5 : 5 : 5 : 4. Antennæ grayish brown, verticils of hairs yellowish white; 16-segmented; segment 1 broad at apical portion, slightly longer than 2, which is globular; 3 to 13 flask-shaped, the diameter of node over twice that of internode; 14 to 16 separated by a short neck, 14 and 15 each with a toothlike projection bearing a small spine at its apical end, segment 16 ovoid with several spinelike hairs at apex. Sensory spines apparently wanting.

Dorsum of thorax light brown, covered with light yellowish gray hairs. Pleura pale brown, apparently without hairs. Wings smoky, with gray hairs, apparently without spots; length over twice greatest width. Base of costa decidedly enlarged. Sc ending at about level of origin of R_1. Fork R_2R_3 at about middle of wing; origin of forking R_2R_3 at the same level with r-m. Fork M_2M_3 much closer to fork R_2R_3 than to r-m. Halteres grayish brown, with short yellowish hairs. Femora of all legs pale brown with yellowish gray hairs; tibiæ and tarsi pale

brown but with yellowish gray hairs. Abdomen entirely pale yellow, covered with thick yellowish gray hairs, especially on the dorsum and hypopygium. Ninth tergite about as long as wide; posterior portion broad. Inferior appendages slender, not well developed basally, but with hairlike spines on their outer surfaces; about twice length of ninth tergite; apically bearing a single spinule which is about one-third length of appendages. Superior appendages 2-segmented; basal segment broad, shorter than the apical, which tapers gradually into an almost pointed end; whole surface of apical segment bearing a few scattered hairlike spines. Ædeagus composed of a single rodlike piece, which splits into halves at about two-thirds its length; each piece supported by a small, triangular, chitinized piece.

Length of body, 1.01 millimeters; wing, 1.09; antenna, 1.17; width of head, 0.44; wing, 0.62.

Female.—Slightly lighter than the male. In venation, markings, and structure of the antennæ like the male. Ventral plate much wider than long, with two distinct lobes posteriorly, bearing long spines and microtrichia. Ovipositor broad basally, tapering gradually to a sharp apex; apical half with several scattered spines.

Length of body, 1.53 millimeters; wing, 1.79; antenna, 1.04; width of head, 0.47; wing, 0.78.

LUZON, Laguna Province, Mount Maquiling, February 23, 1908, reared from wild figs in the forest (*C. S. Banks*); 4 males and 7 females; *8621*, Bureau of Science entomological collection.

PSYCHODA MUSÆ sp. nov. Plate 1, figs. 13 to 16.

Male.—Occiput brown, narrowed medially, covered with erect, grayish white hairs, anterior margin with a single row of from 8 to 10, long, spinelike hairs. Eyes at frons nearly touching each other. Frons and clypeus together not triangular in outline, thickly covered with bushlike grayish white hairs. Clypeus brown, not convex medially, wider than long, whole surface with somewhat recumbent, grayish white hairs. Proboscis brown, reaching to about the middle of the second palpal segment. Tip of labium of medium size, provided with three long teeth and one short tooth; membranous area with two unequal labial spines. Palpi and antennæ of same coloration as clypeus; segments of palpi in the proportion of 13 : 15 : 15 : 20. Antennæ 14-segmented, with whorls of grayish white hairs lying closely to the segments, thus giving it a solid appearance as seen in

Ps. phalænoides; basal segment rather tubular, width less than greatest width of segment 2; which is somewhat spherical, distinctly larger than the other segments; 3 club-shaped, node about as long as its internode; 4 to 12 nearly flask-shaped; 13 slightly constricted at approximately anterior two-thirds, with one small toothlike projection bearing a minute spine; 14 small, ovoid. Sensory spines on segments 3 to 12 with two long anterior branches, one curved basally, at a right angle to the other branch; a slender posterior branch extending a little over the length of its node.

Dorsum of thorax dull brown to bronze; surfaces covered with long whitish hairs. Scutellum somewhat pale brown but with the same coating of hairs as on dorsum of thorax. Pleura pale yellowish brown. Wings over twice as long as broad, slightly angulated at tip. Vestiture of wings smoky. Anterior fringe slightly darker towards base. Sc not well developed; a little over length of the heavily pigmented, semichitinous pad above it. Origin of R_2R_3 distinctly before level of r-m. Fork R_2R_3 before the middle of the wing. Fork M_1M_2 before middle of the wing, closer to fork R_2R_3 than to r-m. Halteres yellowish. All coxæ and trochanters pale brown; femora and tibiæ somewhat darker, with grayish hairs on anterior surface, appearing whitish under reflected light; posterior surface with short and almost white hairs. Anterior surfaces of tarsal segments with dark gray hairs; under reflected light the scalelike hairs appear whitish when seen from above.

Abdomen pale brown dorsally; sides yellowish; venter and hypopygium slightly darker; covering of hairs gray, heavy towards posterior end. Ninth tergite slightly longer than broad, slightly swollen posteriorly. Inferior appendages slender, rather tubular, very slightly swollen basally; terminal spinule short, about one-seventh length of inferior appendages. Basal segment of superior appendages not well developed; distal segment slender, clawlike, with sharp apex, whole surface covered with numerous spinelike hairs. Ædeagus composed of three pieces, a median, broad, somewhat tubular piece with rounded apex and two pointed side pieces, shorter and slenderer than the median, and strongly curved inwardly at their apices.

Length of body, 1 millimeter; wing, 1.09; antenna, 0.73; width of head, 0.27; wing, 0.42.

LUZON, Rizal Province, Alabang, July 10, 1935 (*F. del Rosario*); *19359,* Bureau of Science entomological collection.

This species was collected together with *Ps. phalænoides* and *Ps. alabangensis* from banana stalks at about 0.5 to 1 meter above the ground.

PSYCHODA ALABANGENSIS sp. nov. Plate 1, figs. 17 to 20.

Male.—Occiput light brown, not broad, with gray hairs; a row of five stout hairs on anterior margin. Eyes separated at frons by a distance equal to diameter of one facet. Frontal triangle dull brown, clothed with yellowish gray hairs, these hairs confined to a rectangular area between antennal fossæ. Clypeus dull brown, slightly longer than broad, with long grayish hairs directed toward proboscis. Proboscis brown, with whitish pubescence. Tip of labium provided with three long teeth and two long labial spines. Palpi grayish brown with gray hairs; proportion of palpal segments 15 : 16 : 15 : 19. Antennæ 15-segmented; grayish brown, nodes with long gray hairs distinctly spreading outward; segment 1 slightly longer than 2, which is spherical; 3 to 12 flask-shaped, internode longer than node, except in the first two basal segments; 13 globular, with a neck; 14 smaller than 13 and intimately joined to 13 and 15; segment 15 slightly smaller than 14, ovoid. Sensory spines composed of two, long, anterior branches and one, short, posterior branch.

Dorsum of thorax light brown with erect gray hairs. Wings lanceolate, vestiture gray. Fringes smoky. Sc ending on the same level as origin of R_1. Fork R_2R_3 at about middle of wing; fork M_1M_2 closer to fork R_2R_3 than to r-m. Coxæ, tibiæ, and tarsi of all legs pale brown, with grayish hairs, those on tarsal segments appearing whitish under reflected light.

Abdomen yellowish brown, thickly clothed with hairs similar to those on thorax. Hypopygium pale brown, with gray hairs. Ninth tergite wider than broad, sides swollen; anal flap prominent, covered with short fine hairs and microtrichia. Inferior appendages well developed basally, slightly tapering distally; terminal spinule about one-sixth length of appendages. Basal segment of superior appendages broad and stout, with thick hairs on outer margin; apical segment longer than basal segment, gently curved, tapering to an almost pointed apex and bearing several spinelike hairs. Ædeagus consisting of one rather tubular piece, nearly S-shaped at distal end, posteriorly dividing into two pieces for a short distance, these pieces uniting again posteriorly to form a slender, somewhat pointed piece.

Length of body, 1.11 millimeters; wing, 1.17; antenna, 0.6; width of head, 0.28; wing, 0.34.

LUZON, Rizal Province, Alabang, July 10, 1935 (*F. del Rosario*); *19360,* Bureau of Science entomological collection.

PSYCHODA MANILENSIS sp. nov. Plate 1, figs. 21 to 24.

Female.—Occiput and frontal triangle light brown covered with yellowish hairs. Eyes separated at frons by a distance equal to about four times diameter of one facet. Clypeus pale brown, twice as wide as long, slightly convex, with long grayish hairs. Proboscis brown, bulblike, a little longer than first palpal segment. Paraglossæ with six short teeth on each side of lobe and over twenty long stout spines. Theca heavily chitinized, stem about one-third length of anterior arms. Palpi dark brown, with grayish pubescence, first segment shortest; proportion of segments 2 : 7 : 7 : 9. Antennæ 16-segmented, pale yellow, with long whitish hairs which tend to spread outward; segment 1 stout, slightly longer than 2, which is globular; 3 smaller than 2, with a short neck; 4 to 15 more or less club-shaped, node of each segment longer than internode; 16 elongated and terminating in a spike, with fine microtrichia. Sensory spine composed of one long anterior branch, one on each side of node.

Dorsum of thorax light brown, with long, erect, yellowish white hairs, their tips almost white. Pleura pale brown, without hairs. Wings ovate, somewhat angulated at tip, less than twice as long as wide, large yellowish spot at base of costa close to semichitinous pad, another large yellowish spot at tip of wing extending from tip of R_3 to M_2, entire wing brownish black with distinct black patches at tips of veins R_2, R_3, R_4, R_5, M_1, M_2, and M_3. Sc long, ending at about the same level as origin of R_2R_3. Fork R_2R_3 before level of fork M_1M_2 and before middle of wing. Fork M_1M_2 about as close to r-m as to fork R_2R_3. Knob of halteres yellowish. All femora pale brown, with brownish black hairs mixed with yellowish white hairs. Tibiæ and tarsal segments with brownish black hairs, but distinctly annulated with yellowish white bands.

Abdomen wholly brown, covered with yellowish hairs; incisure lighter. Ventral plate reddish brown, deeply emarginated posteriorly into two rather pronounced lobes. Ovipositor broad at the base, not pointed at apex.

Length of body, 1.5 millimeters; wing, 1.79; antenna, 1.09; width of head, 0.6; wing, 0.96.

LUZON, Manila, June, 1935 (*F. del Rosario*); *19361,* Bureau of Science entomological collection.

PSYCHODA MAKATI sp. nov. Plate 1, figs. 25 to 29.

Male.—Occiput brown, narrowed at sides, clothed with gray hairs. Eyes separated at frons by a distance equal to diameter of one facet. Frons and clypeus together not triangular in outline, covered with gray hairs. Clypeus brown, slightly convex medially, much wider than long, entire surface covered with distinctly gray recumbent hairs. Proboscis brown, longer than first palpal segment. Tip of labium with four fairly long teeth, nearly equal in length; outer surface of membranous area with two, slender, labial spines of about the same length. Palpi and antennæ similar in color to clypeus; proportion of palpal segments 10 : 10 : 11 : 14. Antennæ of sixteen segments; segment 1 of about the same size as 2, which is nearly globular; 3 to 12 flask-shaped, diameter of the node over twice that of its corresponding internode; 13 much broader than 14, but the two segments intimately united with each other; 15 smaller than 14; 16 nearly pear-shaped, separated from 15 by a very short neck. Sensory spines on segments 3 to 13 consisting of two anterior branches and one posterior branch.

Dorsum of thorax light brown, covered with thick-set grayish hairs. Pleura pale brown with a few short, scattered, whitish hairs. Wings over twice as long as broad, covering of hairs smoky. Anterior and posterior fringes slightly darker than other hairs of wing. Sc slightly longer than semichitinous pad above it. Origin of R_2R_3 distinctly before the level of r-m. Fork R_2R_3 and fork M_1M_2 very loosely chitinized. Halteres pale yellow. Coxæ and trochanter of all legs light brown; all femora and tibiæ brown, covering of hairs gray. Tarsal segments with gray hairs appearing yellowish white under reflected light.

Abdomen brown, incisures pale brown. Hypopygium dark brown, covered with thick-set hairs. Ninth tergite wider than broad, slightly swollen medially. Inferior appendages slender, about one and a half times length of ninth tergite; terminal spinule short, wider at tip. Basal segment of superior appendages strongly developed; distal segment well developed, inner margin with a single row of thirteen to fifteen spines. Ædeagus composed of three pieces; a median broad piece slightly curved with rounded apex and two, slender, sharply pointed, side pieces, strongly curved near their apices.

Length of body, 0.94 millimeter; wing, 1; antenna, 0.7; width of head, 0.26; wing, 0.48.

LUZON, Rizal Province, San Pedro Makati, July 4, 1935 (*F. del Rosario*); 1 male and 1 female, attracted to light; *19362*, Bureau of Science entomological collection.

Female.—Resembling male. Occiput much broader than that of male. Eyes more widely separated at frons than those of male. Teeth at tip of labium more prominent. Tip of antenna similar to that of male. Ventral plate narrow near base, widening posteriorly into equal lobes, which are separated by a deep emargination. Ovipositor brown, slightly curved and pointed.

Length of body, 1 millimeter; wing, 1.09; antenna, 0.73; width of head, 0.29; wing, 0.51.

ILLUSTRATIONS

[Drawings inked by Messrs. V. V. Marasigan, J. Trinidad, H. T. Costelo, and R. C. Aguilar and Miss R. R. Ico, of the National Museum Division.]

PLATE 1

FIGS. 1 to 4. *Psychoda baguioensis* sp. nov.; *1*, venation; *2*, male hypopygium; *3*, tip of labium; *4*, tip of antenna, male.

5 to 7. *Psychoda zigzagensis* sp. nov.; *5*, venation; *6*, ventral plate; *7*, tip of labium.

8 to 12. *Psychoda banksi* sp. nov.; *8*, venation; *9*, ventral plate; *10*, tip of antenna, male; *11*, tip of labium; *12*, male hypopygium.

13 to 16. *Psychoda musæ* sp. nov.; *13*, venation; *14*, tip of antenna, male; *15*, tip of labium; *16*, male hypopygium.

PLATE 2

FIGS. 17 to 20. *Psychoda alabangensis* sp. nov.; *17*, venation; *18*, tip of labium; *19*, tip of antenna, male; *20*, male hypopygium.

21 to 24. *Psychoda manilensis* sp. nov.; *21*, venation; *22*, ventral plate; *23*, tip of antenna, female; *24*, tip of labium.

25 to 29. *Psychoda makati* sp. nov.; *25*, venation; *26*, tip of antenna, male; *27*, ventral plate; *28*, tip of labium; *29*, male hypopygium.

TEXT FIGURES

FIG. 1. *Psychoda albipunctata* Williston, female, head in cephalic aspect; *a*, occiput; *b*, frons; *c*, clypeus; *d*, compound eye; *e*, antennal fossa; *f*, antenna; *g*, maxillary palpus.

2. *Psychoda makati* sp. nov., mouth parts, dorsal aspect; *a*, labrum; *b*, hypopharynx; *c*, labium; *d*, galea; *e*, stipite; *f*, palpus.

3. *Psychoda*, five species, showing sensory spines at tip of antennæ of males; *a*, *Ps. olympia* Kincaid; *b*, *Ps. bicolor* Banks; *c*, *Ps. severini* Tonnoir; *d*, *Ps. snowhilli* del Rosario; *e*, *Ps. nigra* Banks.

4. *Psychoda alternata* Say, male hypopygium, dorsal aspect; *a*, ninth tergite; *b*, inferior appendage; *c*, terminal spinule; *d*, superior appendage; *e*, ædeagus; *f*, anal flap.

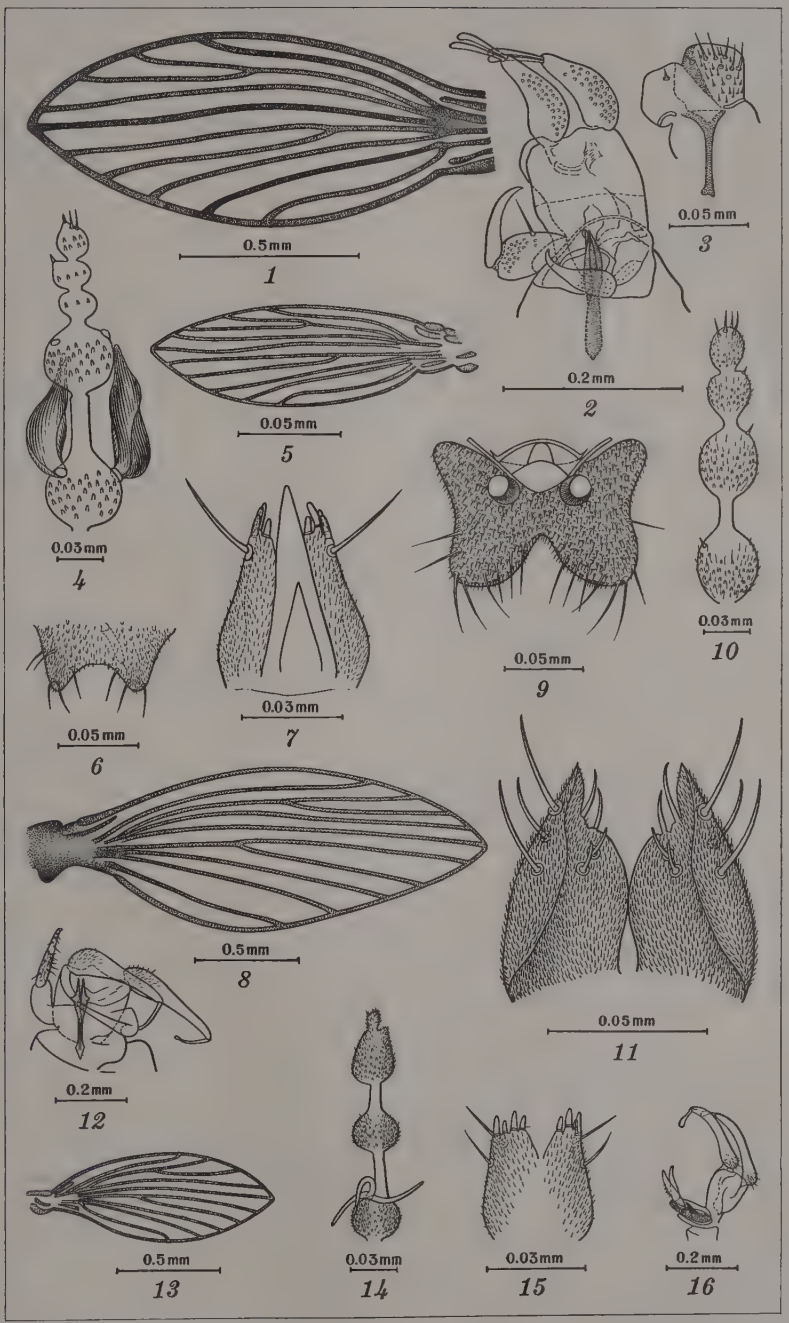

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PLATE 1.

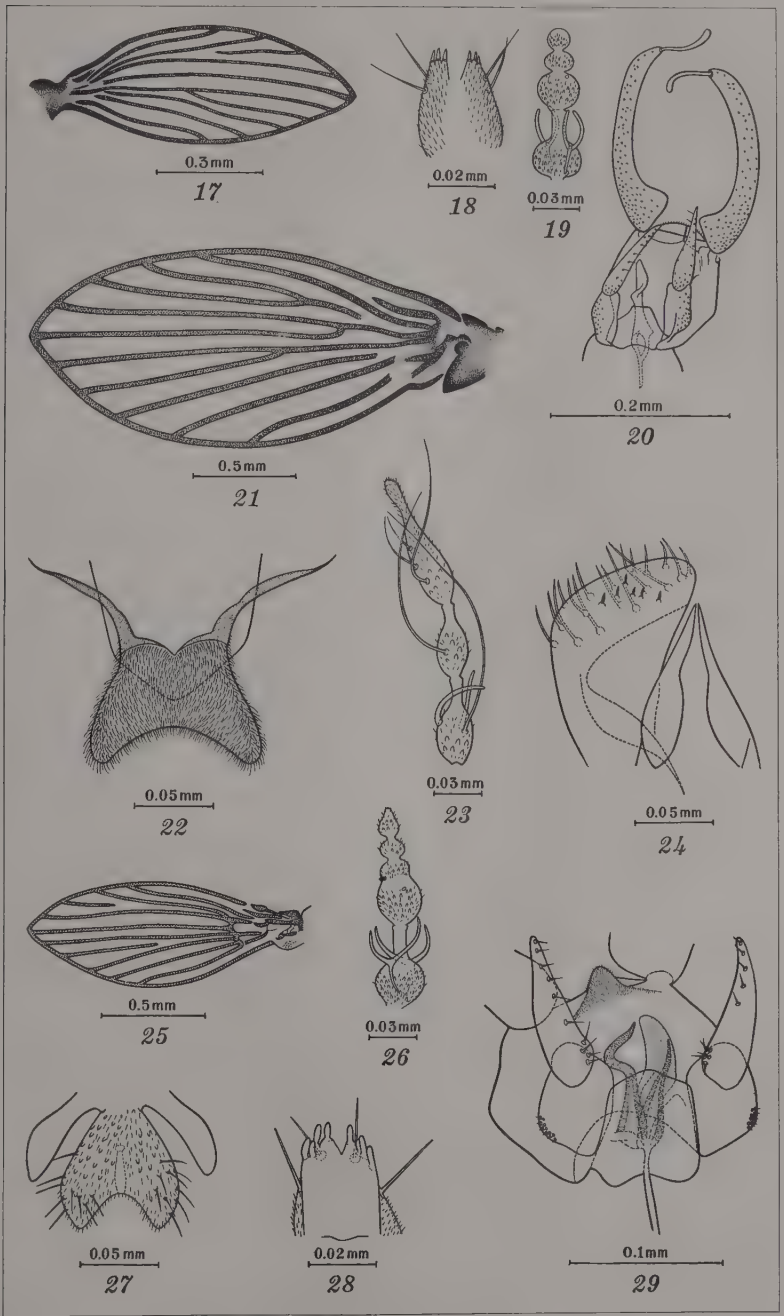
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PLATE 2.

LATE EIGHTEENTH-CENTURY KILN-REJECT JARS EXCAVATED AT THE SAN JUAN DE DIOS HOSPITAL FOUNDATION

By GENEROSO S. MACEDA

Of the National Museum Division, Bureau of Science, Manila

TWO PLATES

INTRODUCTION

Nearly all excavations made in Manila during the last four or five years for the layouts of tall-building foundations have yielded various specimens of archæologic interest. Objects of this nature that have not, or only partly, lost their originality are interesting to archæologists. It is one of these types of material, locally known as *tapayan* (tinaja in Spanish), that is reported in this paper. These tapayans were manufactured in a local kiln and were intended to contain water, vinegar, and other liquids.

It was only during the Spanish régime that the use of kilns and other improvements in pottery making were introduced in the Islands in order to meet the growing demand of the natives.

> The use of kilns and some improvements over the primitive methods of making pottery seem to have been introduced under the Spanish régime in order to meet the demands of household articles not found in use by the natives and to supply earthernware required in certain industries. The use of kilns permitted a better burning of the common red ware and they produced a sufficiently high temperature to semivitrify the product.(1)

Of course, there were already methods employed by the natives, but they were primitive ones, and the output was crude and only for home use.

Other places where collections of numerous Chinese, Siamese, and European porcelain pieces, native pottery, and human bones have been made are the Post Office site, the Metropolitan Theater site, the Ideal Theater site, the Plaza Moraga site, the Heacock Building site, and the Great Eastern Hotel site. The specimens are now in the collections of Prof. H. O. Beyer, of the University of the Philippines, and in the Bureau of Science; while Dr. J. P.

Bantug, of the Bureau of Health, and several other collectors have fair-sized collections from specific places.

THE PRESENT SITE

The jars were excavated at the site of the rear portion of the San Juan de Dios Hospital building, bordering Legaspi and San Francisco Streets. These jars were inverted in rows beneath a layer of earth and débris 1 meter thick, and practically covered an area of approximately 150 square meters.

JARS AS KILN REJECTS

The jars found were rejects with cracks on the sides and some with deformed shapes due either to improper heating or overcrowding in the kiln. Such jars cannot be utilized as containers. These jars probably were the waste products of one of the kilns owned by the religious orders in San Pedro Makati, Rizal Province, Luzon, from which this religious hospital obtained the rejects through donation.

> . . . the religious orders possess the following estates: . . ., San Pedro Macati (where they have their earthenware factory, from which they make annually thirty thousand pesos fuertes net profit).(2)

DESCRIPTION OF THE JARS

The original color and luster have almost been preserved. The jars are heavy and of hard body. The shape is like that of the Chinese Sung type jars. The diameter is small, while the color is dark reddish brown and very similar to that of the ordinary tapayan in the Manila markets. The jars were not originally broken due to pressure of the ground floor (Plate 1), but were intentionally cracked by laborers in the belief that they contained hidden treasures.

USES OF THE JARS IN RELATION TO BUILDING CONSTRUCTION

The practical uses of the jars in connection with building construction may be mentioned as follows: The jars were laid in rows to support the basement floor of the hospital building; they took the place of the tremendous amount of earth that otherwise would have been needed to raise the elevation of the ground floor; and they were placed in such a manner in order to lessen the capability of the ground to hold water. The third use is rather an ingenious way of constructing floor tiles. The water that was occasionally left by the esteros during high tide

in those days easily seeps through this construction, which is porous on account of the spaces between and inside the jars.

POSSIBLE DATING OF THE JARS

The determination of the date of the manufacture of these jars depends on two factors; namely, the history of the hospital and that of the kilns where the jars were made.

We may first attempt to determine the date when the Hospital de San Juan de Dios building was constructed. This will give us the nearest age of the jars.

By virtue of a royal decree August 17, 1865, the Archbishop and the Governor-General transferred the management of the said hospital to a Board of Directors composed of *Excmo. Sr. Regente de la Real Audencia, presidente; Don* Tomas Balbas y Castro, *consejero de Administración, tesorero; Don* Mariano Tuason, *Don* Francisco de Paula Cembrano, *Don* Joaquin Pardo de Tavera, *consejeros de Administración;* y *Don* Manuel Asensi, *ponente de la Sección de lo Contencioso del mismo Consejo,* y *Don* Casimiro Cortazar, *jefe de Session del Gobierno Superior Civil.* This board of directors held a *Bazar de Caridad* in 1867 and out of this they made a net profit of 29,652.11 dollars. November 18, 1867, the *Excmo. Sr. Gobernador General Don* Jose de la Gandara signed the act of construction and laid the cornerstone of the recently demolished (rear portion only) San Juan de Dios Hospital building. The following day, November 19, 1867, the construction began.(3)

This date does not furnish us the exact age of the jars, but since these jars were used as filling material of the ground floor of the building it can be safely asserted that the age of these jars is not less than 68 years. However, these jars were not manufactured intentionally for filling purposes, but they were kiln wastes, and became such presumably some years before the hospital building was constructed. Hence, these jars are obviously older than the present Hospital de San Juan de Dios building.

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2. Pardo de Tavera, T. H., in Blair and Robertson's Philippine Islands 1493–1898 50 (1907) 154.
3. Giner, Gregorio Sanchez. Memoria Historico-Administrativa del Hospital de San Juan de Dios (1897) 17–19.

ILLUSTRATIONS

[Photographed by Dr. C. S. Angbengco and Mr. G. Panlillo, of the Bureau of Science.]

PLATE 1

FIG. 1. The excavated foundation of the San Juan de Dios Hospital building showing the kiln-reject jars used as partial fillers.

2. Another portion of the excavated foundation of the same hospital showing the cracked jars, which were intentionally broken by the laborers in search for treasure.

PLATE 2

FIG. 1. Four representative types of the kiln-reject jars.

a. Greatest diameter, 15⅞ inches; height, 21⅞.

b. Greatest diameter, 16½ inches; height, 22½.

c. Greatest diameter, 17⅛ inches; height, 24¾.

d. Greatest diameter, 18¼ inches; height, 21¼.

2. Interior view of one of the kilns in San Pedro Makati, Rizal Province, Luzon, introduced by the Chinese during the Spanish regime. The proprietor of the kiln is Mr. Justino Jimenez, of the same town.

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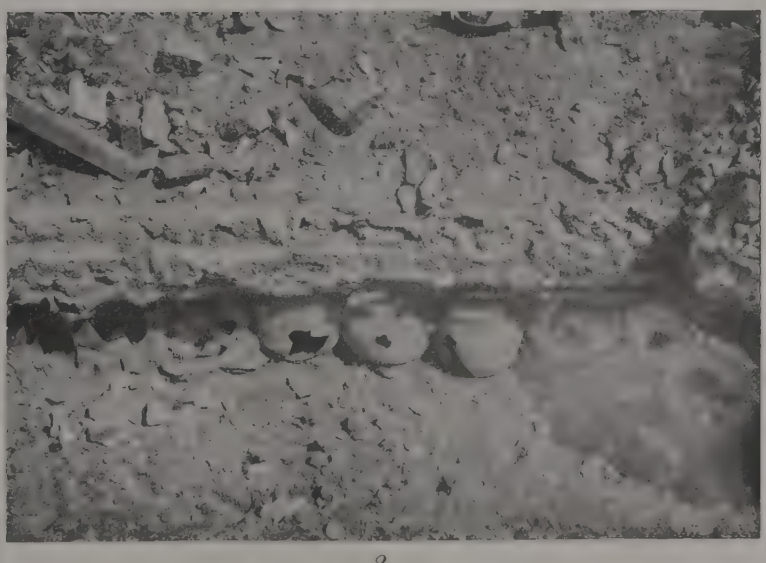

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PLATE 1.

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PLATE 2.

BOOKS

Acknowledgment of all books received by the Philippine Journal of Science will be made in this column, from which a selection will be made for review.

RECEIVED

American association of cereal chemists. Committee on methods of analysis. Cereal laboratory methods with reference tables, comp. by Committee on methods of analysis. 3d ed. completely rev. Omaha, Nebraska, American association of cereal chemists, 1935. viii, 204 pp. illus.

American society for testing materials. Proceedings. 38th, 1935, pts. I–II, Philadelphia, Pa., American society for testing materials, 1935. Illus., fold. tables, diagrs. Price, $8.80.

BARNARD, W. G. The technique of a post-mortem examination. London, London county council, 1935. Cover title, 7 pp. Price, $0.25.

BELLER, SAMUEL. Coleoptera of Washington: Chrysomelidae, by Samuel Beller and Melville H. Hatch. Seattle, Washington, University of Washington press, 1932. 76 pp. plate. (Washington University. Publications in biology, 1932, vol. 1, No. 2, pp. 65–144.) Price, $0.50.

BESSEY, E. A. A text-book of mycology. Philadelphia, P. Blakiston's son & co. [c. 1935]. xv, 495 pp. illus. ports.

FICHERA, G. Chemioterapia del cancro. Milano, U. Hoepli, 1934. 213 pp. tables, plates. Price, $2.50.

GIBBS, C. S. A guide to sexing chicks. N. Y., Orange Judd pub. co., 1935. 63 pp. illus. Price, $1.25.

HATCH, M. H. Coleoptera of Washington: Silphidae, by Melville H. Hatch and William Reuter, jr. Seattle, Washington, University of Washington press, 1934. 13 pp. (Washington University. Publications in biology, 1934, vol. 1, No. 3, pp. 147–162.) Price, $0.15.

HOTSON, J. W. Key to the rusts of the Pacific northwest. Seattle, Washington, Univ. of Washington press, 1934. (Washington University. Publications in biology, 1934, vol. 3.) 103 pp. illus. Price, $1.50.

HUBBS, C. L. Description of two new American species referable to the rockfish genus Sebastodes, with notes on related species, by Carl L. Hubbs and Leonard P. Schultz. Seattle, Washington, University of Washington press, 1933. (Washington University. Publications in biology, 1933, vol. 2, No. 2, pp. 15–44.) 42 pp. table, plates. Price, $0.25.

JAFFE, BERNARD. New world of chemistry. N. Y., Silver Burett and Co. [c. 1935] xii, 566 pp. xxx, front. illus., port., diagrs. Price, $1.80.

James Johnstone memorial volume. Liverpool, London, University press, 1934. x, 348 pp. front. illus. tables, fold. diagrs. maps. Price, $5.25.

JOHNSON, M. W. Seasonal distribution of plankton at Friday Harbor, Washington. Seattle, Washington, Univ. of Washington press, 1932.

(Washington University. Publications in oceanography, 1932, vol. 1, No. 1, pp. 1–38.) 38 pp. tables, diagrs. Price, $0.35.

JOHNSON, M. W. The seasonal settlement of shipworms, barnacles, and other wharf-pile organisms at Friday Harbor, Washington, by Martin W. Johnson and Robert C. Miller. Seattle, Washington, Univ. of Washington press, 1935. (Washington University. Publications in oceanography, 1935, vol. 2, No. 1, pp. 1–18.) 18 pp. tables, diagrs. Price, $0.20.

KENDEIGH, S. C. The rôle of environment in the life of birds. 1934. 117 pp. maps. tables, diagrs.

PHIFER, L. D. Phytoplankton of East Sound, Washington, February to November, 1932. Seattle, Washington, University of Washington press, 1934. (Washington University. Publications in oceanography, 1934, vol. 1, No. 4, pp. 97–110.) 12 pp. tables, diagrs. Price, $0.15.

PHIFER, L. D. Vertical distribution of diatoms in the Strait of Juan de Fuca. Seattle, Washington, University of Washington press, 1934. (Washington University. Publications in oceanography, 1934, vol. 1, No. 3, pp. 83–96.) 12 pp. tables, diagrs. Price, $0.15.

SEWART, DOROTHY. Comp. The medical cookery book, containing three hundred recipes for the use of invalids, convalescents and children, comp. by Dorothy Sewart, with an introduction by J. W. McNee. Bristol, J. Wright & sons, 1935. 136 pp. Price, $1.25.

SHOEMAKER, J. S. Small-fruit culture; a text for instruction and reference work and a guide for field practice. Philadelphia, P. Blakiston's son & Co. [c. 1934]. xv, 434 pp. illus., tables. Price, $3.50.

SLAGLE, E. C. Games and field day programs, comp. and ed. by Eleanor Clarke Slagle. Utica, N. Y., State hospital press [c. 1933]. vi, 134 pp. illus.

WORLEY, L. G. The spiders of Washington; with special reference to those of the San Juan Islands. Seattle, Washington, University of Washington press, 1932. (Washington University. Publications in biology, 1932, vol. 1, No. 1, pp. 1–63.) 63 pp. Price, $0.50.

REVIEWS

Practical Handbook on Electro-Plating. W. Canning & Co., Ltd., Birmingham, London, 12th ed., 1934. 344 pp. Price, $1.

This book gives a detailed description of the operations carried on in an electroplating shop. It is profusely illustrated with drawings and photographs of numerous electroplating installations indicating the arrangement of the apparatus and accessories used. The contents of the book are divided into four sections. Section 1 deals with the grinding and polishing of the object to be plated; section 2, with cleaning and dipping operations; section 3, with electroplating; section 4, with bronzing, lacquering, and enameling.

The detailed descriptions in the text together with the photographic illustrations of the methods of carrying out the various

operations give the ordinary reader complete information on the subject.—F. D. R.

Southeast of Zamboanga. By Vic Hurley. E. P. Dutton & Co., Inc., New York, 1935. 237 pp. Illustrated. Price, $3.

This is a book of travel or forest adventure. It deals with two Americans who, after graduation from college, went to southeastern Mindanao to make a fortune by raising coconuts. During their stay in that part of Mindanao they encountered great hardships and became sick for they were not accustomed to the climate.

The book covers a period of one year in the interior of Mindanao and deals with the economic and social life of the Moros and other pagan groups. It portrays the economic and social life of the people at close range. It is exceedingly interesting and inspires an anthropologist to make further observations on the Mohammedan and pagan peoples in that part of Mindanao where their economic and social life is least known.—R. E. G.

The Elements of a National Mineral Policy. Prepared by The Mineral Inquiry: C. K. Leith, Chairman. American Institute of Mining and Metallurgical Engineers, New York, 1933. 162 pp.

This book gives a brief but comprehensive discussion of a few selected topics related to the mineral industry and the place of that industry in the newly evolved philosophy of economic nationalism and the problem of national defense. There is a great divergence of opinion among several authorities as to the national policy a nation should pursue in order to attain economic self-sufficiency and national security. It may be noted, however, that there is a tendency to favor the coördination of all industries with the view of producing the best economic results for the nation as a whole as against the present catch-as-catch-can practice; that is, everybody for himself with private gain as the supreme aim of his endeavors.

While the book propounds no definite policy with regard to minerals, it is nevertheless very enlightening and should be valuable to students of economics, especially to those who are at the helm of the Commonwealth of the Philippines and are called upon to direct the destiny of our newly-born nation.—Q. A. A.

Biology for the Public School Administrator. By Frederick L. Fitzpatrick. Teachers College, Columbia University, New York, 1st ed., 1934. 87 pp.

This book is intended primarily for administrators who have to deal with the framing of courses in general biology for high

schools. The subject, however, is so presented as to be useful for student teachers, teachers, critic teachers, and supervising teachers in high-school biology. Concisely written, it gives a historical sketch of the development of the high-school course in general biology and sets forth the necessity of correlating the course with everyday experiences. It enumerates the qualifications, the training, and the experience a biology teacher should have. It also gives the various teaching procedures in general biology as well as the necessary supplies and equipment needed for the successful teaching of biology. Chapter II of the text is an outline of the course in general biology, which is so complete, comprehensive, and inclusive that it could be very well adopted in the Philippines. The list of references and other sources of information contained in the last chapter is a good aid to teachers and investigators in the preparation of lessons or manuscripts.—H. A. R.

Population Theories and their Application, with Special Interest to Japan. By E. F. Penrose. California Food Research Institute, Stanford University, 1934. 347 pp. Price, $3.50.

Population Theories and their Application is an offshoot of some studies undertaken by the author under the Food Research Institute established at Stanford University, California.

It deals with the economic situation which has developed in modern times with the passing of regional self-sufficiency and the large increase in the population of eastern and southeastern Asia.

The rapid growth of population has not prevented changes which have transformed the material conditions of life in modern times.

Inventions, improvements in technic, expansion in productive capacity, and the exercise of more individual control over the birth rate have made it increasingly improbable that the world as a whole will ever be placed in the situation envisaged in the Malthusian theory.

The human race cannot in any case be distributed over the world in such a way that each group has the same advantages as all others with respect to the possession of natural resources. Inequalities can to a certain extent be offset by movements of population, capital, and goods.

It is an up-to-date book because it deals with the present economic conditions of life particularly in eastern and southeastern Asia.—R. E. G.

Sex and Temperament; In Three Primitive Societies. By Margaret Mead. W. Morrow and Company, New York, 1935. 335 pp. Price, $3.

The author of this book of descriptive anthropology is assistant curator of ethnology in the American Museum of Natural History. The materials accumulated in the book are the result of two expeditions to New Guinea in 1931–1933.

The book deals with the social life of the three primitive people of New Guinea—the mountain-dwelling Arapesh, the river-dwelling Mundugumor, and the lake-dwelling Tchambuli.

By studying these three types of primitive peoples the author is led to suggest that the behavior which our society believes is determined by sex, may be socially determined, and that the most significant differences are those between different human beings and not between the sexes.

The book contains fresh and abundant data concerning the peculiar social life of the three primitive peoples mentioned, into the social life of whom the author displays a keen insight.

—R. E. G.

Manual of Land and Fresh-Water Vertebrate Animals of the United States (exclusive of Birds). By Henry S. Pratt. P. Blakiston's Son & Co., Inc., Philadelphia, 2d ed., 1935. 416 pp. Price, $6.

This book is a compilation of the works of various authors on the identification, habitat, and geographical limits of species, subspecies of vertebrate animals, exclusive of birds, of the whole United States between the Canadian and Mexican borders. The second edition embodies numerous changes in the nomenclature of the vertebrate animals of the United States and defines more precisely their known ranges of distribution. This book is important to students of zoölogy, especially to those taking up work in animal geography. Although the species of animals treated are non-Philippine, this book should interest Philippine students of comparative anatomy and geographical distribution of vertebrates as it gives much useful information on the characters used in the identification of fishes, amphibians, reptiles, and mammals. The book is fortunately provided with comprehensive keys for the determination of the higher groups.

—H. A. R.

Handbook of Industrial Fabrics. By George B. Haven. Wellington Sears Co., New York City, 1934. 538 pp. Price, $2.

One finds in this authoritative work by the head of the textile research department of the Massachusetts Institute of Techno-

logy an abundance of information and data on industrial fabrics, that, having been hitherto in more or less scattered form, have been brought together and condensed for the convenience of textile chemists, engineers, designers, testing laboratories, and students. The book is presented in a concise manner and is comprehensive enough to include fibers and fabrics of cotton, cotton yarns, uses of fabrics and their properties, laboratory design and practice, and specifications and test methods. It is primarily intended as a handbook for the technical worker, but will be equally useful as a reference volume for others.—J. C. E.

Modern Method of Birth Control. By Thurston S. Welton. W. J. Black, Inc., New York City, 1935. 168 pp. Price, $3.

Doctor Welton has gathered together in more accessible and companionable form the results of long years of investigation conducted by authorities on drugless birth control. The separate labors of Doctor Ogino, of Japan, and Doctor Knaus, of Australia, represent the most authoritative efforts ever pursued by scientists along this line and they are here conjoined for the benefit of prolific householders.

The book addresses especially itself to dogmatic and faithful members of the Roman Catholic faith who will find a relief from excess biological procreation in a manner sanctioned by the Holy Father at Rome, without undergoing the strain of denying the faithful the essential fulfillment of the natural biological urge. This book advocates, therefore, the practice of rhythm.

The studies of the above-cited authorities point out that within the mense cycle of women there are two fallow, or sterile, periods and between these two periods is the fertile one, representing only a week of virtual fasting. It could be made less by three days if women have no aversion to douching after cohabitation if such a thing happen just before the advent of the real fertile period.—M. G. C.

The Marine Plankton with Special Reference to Investigations Made at Port Erin, Isle of Man, during 1907–1914. By James Johnstone, Andrew Scott, and H. C. Chadwick. The University Press of Liverpool, Ltd., London, 3d reprint, 1934. 194 pp. Price, $3.25.

This text, written by outstanding English authorities on oceanography and marine biology, is one of the best books put out on the subject. The contents are the results of intensive and extensive plankton studies for a great number of years. In a very readable and clear form, the authors have set forth the

categories of life in the sea, the various plankton types, the distribution of the plankton, and the relation between organic production and the plankton. In an appendix the various types of plankton nets and methods of collection, as well as the methods of the estimation of the catch, are accurately described. The book is practical as well as theoretical. Students requiring the identification of their plankton can readily use its many plates and descriptions for reference. Once the organisms are identified the student gets much information from the many tables and the text as to abundance and seasonal occurrence. The more philosophic biologist can follow Professor Johnstone's inquiry into the causes and effects of marine phenomena. This book is indispensable for students as well as researchers in marine plankton.—H. A. R.

American Scientists. By C. J. Hylander. Macmillan Company, New York, 1935. 186 pp. Price, $2.

Narratives of the lives of great men usually tend to have an inspiring influence for, as Longfellow said:

> Lives of great men all remind us
> We can make our lives sublime,
> And, departing, leave behind us
> Footprints on the sands of time.

Books of this kind record the great things men have done, but many of them do not state exactly how these men got their start on the road to fame. This book is different in this respect. For instance, it states how Roy Chapman Andrews began his scientific career by washing floors and receiving no salary for it. This is quite a contrast to many of our present-day graduates who are more concerned with the salary they can get than with their work as a career.

American Scientists is a little book that is easy to read and is very interesting not only for scientists but also for the layman.—A. P. W.

Helminth Parasites of the Domesticated Animals in India. By G. D. Bhalerao. (The Imperial Council of Agricultural Research. Scientific Monograph No. 6.) Manager of Publications, Delhi, 1935. 365 pp. 13s. 3d.

In view of the economic importance of worm parasites in relation to the health of domesticated animals in India, the author deplores the lack of reference facilities for the correct determination of helminths and the inconvenience of sending

collections to foreign specialists. The present volume has been prepared to fill this gap and serves at the same time as a systematic check list of the helminth parasites reported from Indian domesticated animals. It should be a useful reference to workers, not only in India but in other countries as well.—M. T.

Sedgwick's Principles of Sanitary Science and Public Health. By Samuel C. Prescott and Murray P. Horwood. The Macmillan Company, New York, rewritten & enlarged, 1935. 654 pp. Price, $4.25.

This book gives important information about health and its preservation. It begins with the relation of the intrinsic mechanism of the human machinery to the innumerable extrinsic factors of the human body. The authors formulate fundamental facts for the preservation of the individual and the community. They also tell how to conquer the vast enemies of human life in particular and the community in general. Therefore, this book is recommended not only to students and health practitioners, but to all those interested in sanitation and public health.—M. B.

Cereal Laboratory Methods with Reference Tables. Compiled by The Committee on Methods of Analysis, American Association of Cereal Chemists. The Association, Omaha, Nebraska, 3d ed., 1935. 204 pp.

This is the revised third edition of Cereal Laboratory Methods. It contains a number of procedures not given in the Methods of Analysis of the Association of Official Agricultural Chemists. The analytical procedures are written up plainly and concisely. This book is a valuable addition to the literature on cereal chemistry. It should also serve as an important reference book for general analytical work.—A. J. H.

A. S. T. M. Standards on Rubber Products. Prepared by Committee D–11 on Rubber Products. A. S. T. M., Philadelphia, Penn., 1935. 204 pp.

This compilation is in a convenient form for laboratory use and a valuable one for reference purposes. The specifications include detailed procedures for physical and chemical examinations of such products as cotton rubber-lined fire hose, rubber pump valves, friction tape, rubber insulating tape, rubber gloves, rubber matting, rubber insulating blankets, and insulating wire and cable.—R. H. A.

Modern Motherhood; A Book of Information on Complete Maternity Care: Prenatal-Delivery-Aftercare. By C. E. Heaton. Farrar & Rinehart, Inc., New York, 1935. 271 pp. Price, $2.

This book gives to expectant parents sound information on the nature of pregnancy and childbirth and though tactfully discouraging self-doctoring, gives full information on maternity care. In easy, comprehensible language and breathing throughout a wise conservatism supplemented by opportune quotations, the author discusses the subject of motherhood from the strictly biological to the psychological, economical, and sociological aspects. Not only prospective parents but other intelligent readers of both sexes should benefit by a careful perusal of this book.

—A. V. C.

Le Chauffage par les Combustibles Liquides. Par A. Guillermic. Librairie Polytechnique Ch. Beranger, Paris, 1935. 394 pp. 338 figs.

This book gives a description of the various fuel oils generally used in the industries. It discusses various types of oil burners, illustrated by appropriate drawings. It includes those that are suitable for operation with low and high air pressure, steam, and atomization by mechanical means. Perhaps the most important feature of the book is the drawings of actual installations as used in the industries and the comparative results obtained on the use of fuel oil and coal for heating and power.

—F. D. R.

Child Nutrition on a Low Priced Diet. By Mary S. Rose and Gertrude M. Borgeson. (Child Development Monographs, no. 17.) Teachers College, Columbia University, New York City, 1935. 109 pp. Price, $1.50.

This monograph gives instructive information on the nutrition of children. It is very practical, since the nutritious diets suggested are low in price. The discussions of the various topics such as diet, physical development, etc., are given in a popular style and hence the monograph is very appropriate for the layman.—A. J. H.

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